NAPPO
North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

4. Host-Specificity Testing:

4.2 Laboratory Tests

4.3 Information from area of origin

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Outline: determining host specificity

- Background
- Scientific methods
- Experimental approaches
- Experimental design and analysis
- Pitfalls in statistical analysis and interpretation




NAPPO RSPM 12 requires that host specificity be determined from laboratory tests and from information from the are of origin (Sections 4.2, 4.3)

NAPPO Regional Standards for Phytosanitary Measures (RSPM)

RSPM 12
Guidelines for Petition for First Release of Non-indigenous Entomophagous Biological Control Agents

Date

The Secretariat of the North American Plant Protection Organization
1431 Merivale Road, 3rd Floor, Room 140
Ottawa, Ontario, Canada K2B 0B9

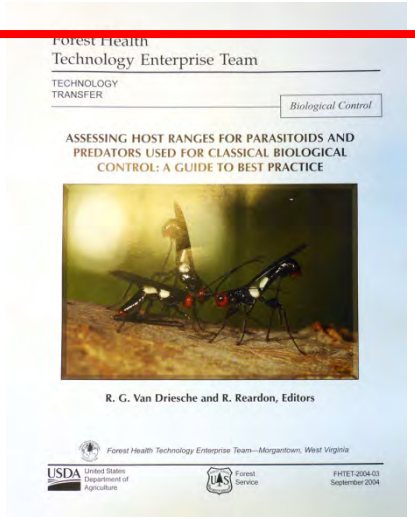
Some key words in the standard:

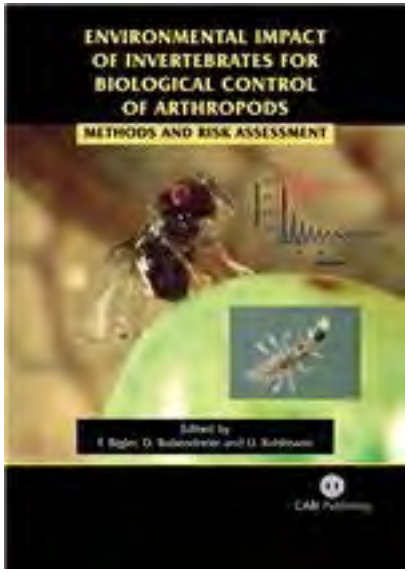
4.2 Laboratory tests (**replicated** no-choice and choice feeding **tests**, oviposition tests, development tests), including information on offspring survival, sex ratio, and fecundity. **Include positive controls** ~~where feasible~~.

4.3 Information on the biological control agent from the area of origin based on **field surveys or experimental field manipulation** as feasible.

Tools and principles

- **Van Lenteren et al.** (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl* 48, 3-38.
- **Van Lenteren et al.** (2006). Assessing risks of releasing exotic biological control agents of arthropod pests. *Annu. Rev. Entomol.*, *51*, 609-634.
- **Bigler, F. Babendrier, D., and Kuhlmann, U.** (2006)Environmental Impact of Invertebrates for Biological Control of Arthropods. CABI Publishing.
- **Van Driesche, R., & Reardon, R. C.** (2004). Assessing host ranges of parasitoids and predators used for classical biological control.





Host specificity testing should:

- provide as accurate an estimation as possible of the fundamental and realized host ranges of the agent (this attack is “necessary” for non-target impacts); and
- provide an estimate of the risk to non-targets (might this attack be “sufficient” for negative impacts?); and
- provide a foundation for follow-up research to estimate the risk to non-targets under field conditions if appropriate, or to conduct post-release surveys.

A definition

- A non-target species is a host if, by attacking the species, an individual parasitoid increases its fitness – i.e., the probability that one or more female offspring survives to mate and reproduce
- A non-target species is a prey if, by attacking the species, an individual predator increases its fitness – i.e., the prey increases the probability that one or more female offspring be produced and will survive to mate and reproduce

1. Ask the right question!

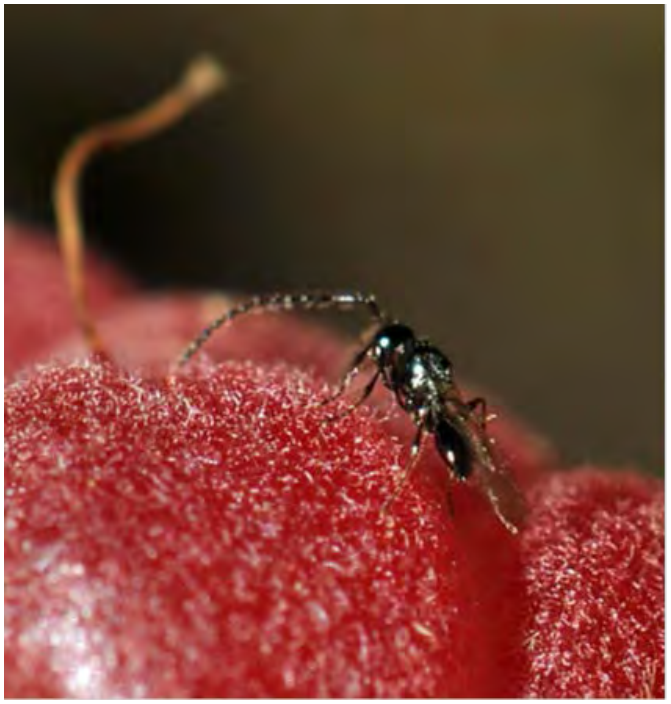
"If the outcomes of purposeful introductions, including those of classical biological control and genetically engineered organisms, are to become predictable, we must ask the right questions and gather appropriate data to answer them (88, 94) "

Howarth, F.G. (1991) Environmental impacts of classical biological control. *Annu. Rev. Entomol.* 36, 485-509.

Ask the right question in the right way!

"By performing an experiment, it remains impossible to prove, for example, that a natural enemy will never attack a non-target host or prey."

Hoffmeister, T.S., et al. (2006) Statistical tools to improve the quality of experiments and data analysis for assessing non-target effects pp. 222-240 in Bigler, F, et al. (eds). Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment.



Why determine host specificity?

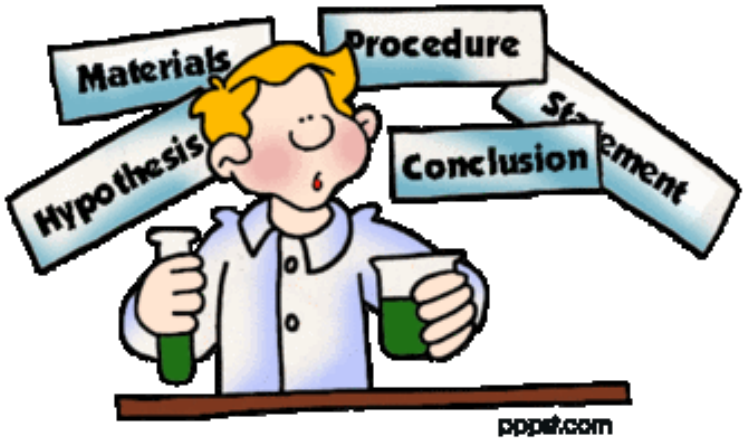
Data from these experiments and surveys provides the background that is necessary to make informed comments on the requirements 5.3, 5.4 and 5.5 which relate to the potential impacts of the agent on organisms in North America.

An obvious constraint:

- One cannot test all potential non-target organisms.
- The choice of non-target test species is crucially important as the behaviour of the agent toward these species becomes the predictor of agent behaviour when released into the environment.

The scientific method

- Hypotheses must be **testable** and **falsifiable**
- Phenomena must be **observable** and **measurable**
- The results must be **rational** and **predictable**
- The experiments must be **reproducible**



Alternate and Null Hypotheses

- The [response] on non-target organisms is different from the [response] on target organisms (the hypothesis of interest - but not falsifiable, therefore an alternate hypothesis)

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- The [response] on non-target organisms is not different from the [response] on target organisms (the null hypothesis, testable and falsifiable)

An example:

- *Peristenus digoneutus* will not attack any host except lygus bugs (an hypothesis of interest, but not testable)
- *Peristenus digoneutus* will attack *Amblytylus nasutus*, *Leptopterna dolabrata* and *Melanotrichus coagulatus* at the same rate as lygus bugs (the null hypothesis, testable and falsifiable)

What is a positive control?

- These are experimental controls that demonstrate that the agents are performing as intended.
- In host specificity testing, the appropriate positive control is the target host.
- Note that negative controls, for example, mortality of subjects in the absence of the natural enemy, may also be needed, depending on the system

What to measure?

- Parasitoids
  - Attack/oviposition rate
  - Successful emergence
  - Number killed
  - Development time
  - Size of offspring
  - Sex ratio of offspring
  - …

  - RELATIVE TO THE TARGET HOST (POSITIVE CONTROL)

What to measure?

- Predators
  - Number attacked
  - Number consumed per unit of time
  - Predator survival
  - Number of eggs laid
  - Offspring developing
  - Development time
  - Size of offspring
  - …

  - RELATIVE TO THE TARGET HOST (POSTIVE CONTROL)

The science of host specificity testing

- Hypotheses must be **testable** and **falsifiable**
- Phenomena must be **observable** and **measurable**
- The results must be **rational** and **predictable**
- The experiments must be **reproducible**

Complications that need to be controlled, described and accounted for

- Enemy state (attack is conditional on state)
  - Age, fecundity, gut fullness (predators), host supply, experience…
- Host/prey state (attack is conditional on state)
  - Age of host, nutritional state, disease…
- Context
  - host plant odours, architecture, surfaces, distractions,…
- Abiotic environment
  - temperature, RH, light, time of day, time of year, weather …
- These can be incorporated as effects in statistical models

Complications which are difficult to control, but nonetheless must be accounted for

- Agent supply may constrain experimental design
  - These issues can also be dealt with in statistical models
- Non-target species may be difficult to find/rear/maintain in suitable condition
  - Therefore you need suitable proxies
- Threatened and endangered non-targets likely cannot be tested (5.5).
- Suitable facilities may not be available
- Research is expensive

The science of host specificity testing

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EACH host specificity test should stand the test of peer review

- Introduction - hypothesis, rationale for test
- Materials and methods – a complete and clear account of experimental design, apparatus, conditions, states, history of organisms, replication and blocking, analysis approaches and rationale, and more.
- Results – a complete reporting of the results, with a variance measure (standard error, 95% CL) for every mean; all statistical analyses reported with models and N; electronic versions of data should be available;
- Discussion/conclusions – interpret the results in the context of your hypothesis, and previous studies.

Experimental approaches for host specificity testing

- Laboratory experiments in the native range
  - Provides knowledge of the host/prey range of the agent in its native range
  - Phylogeny, ecological associations provide the necessary background for introduced range studies

Experimental approaches for host specificity testing

- Quarantine lab experiments in introduced range
  - Essential to gauge how the agent might use the non-target species in the introduced range
  - Access to non-target species (possible hosts/prey) is generally not possible otherwise

Experimental approaches for host specificity testing

- Field surveys and experiments in native range
  - These can be very important to determine
    - timing of the agent relative to possible hosts,
    - dispersal of the agent from release points,
    - distribution of the agent in different habitats
  - Non-target host/prey exposures where agent is present can help to predict likely host range under natural conditions

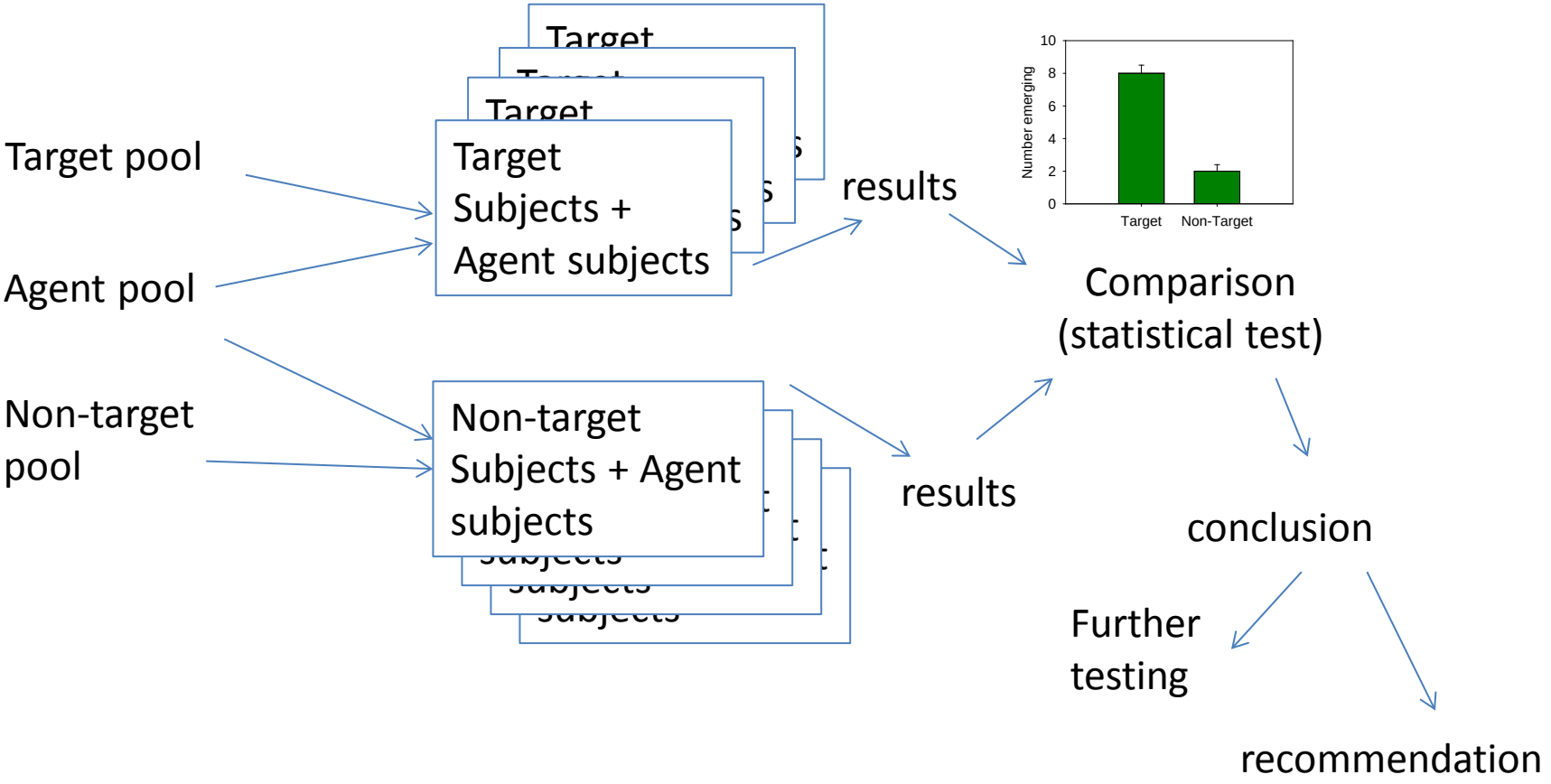
Experimental approaches for host specificity testing

- Field experiments in the introduced range
  - These are generally not possible until post-release.

The NAPPO standard requires

- Laboratory tests (**replicated** no-choice and choice feeding tests, oviposition tests, development tests), including information on offspring survival, sex ratio, and fecundity.

Tests are scientific experiments:



Attack/oviposition

- No-Choice tests
  - The agent is provided with either the non-target, or the target (positive control).
  - The agent can either attack the provided host, or not (a binomial decision)
  - Negative results can be robust if properly designed and replicated sufficiently
  - can be highly affected by state and experience
    - Naïve/experienced, deprived/starved/fed, young/old, …
  - Can therefore be very difficult to interpret

Attack/oviposition choice tests an opinion

- Choice tests are not very meaningful and impossible to interpret ***with respect to host specificity***
- ***The concept of choice is anthropomorphic***

Attack/oviposition

- The sole purpose of attack/oviposition studies is to determine which species could possibly be in the host range
- These studies do not establish host specificity or host range

Oviposition and Development

- Experiments under controlled conditions
- Parasitoids:
  - attacks that produce offspring; development time;
- Predators:
  - Survival of adults on diet of non-target
  - Production of eggs on diet of non-target
  - Development of eggs to adult on diet of non-target
- These establish the fundamental host range

Fundamental host/prey range

- The species which the agent can successfully complete development in/on or
- The species which the agent can successfully kill and eat.

In many circumstances, explicit attack/oviposition studies can be skipped:

1. Draw agents from the pool,
2. Expose them to either targets or non-targets
3. Record attacks (this is just extra information)
4. When there is attack, hold these hosts and determine survival and quality of offspring.
5. Measure also, for example, development time, size of offspring, sex ratio

Predators are really difficult

- Feed the predator exclusively on a diet of non-targets?
  - Determine development or reproduction?
- Feed the predator on mixed diets?
- Functional response to the non-target across a gradient of abundance within target prey populations
- or

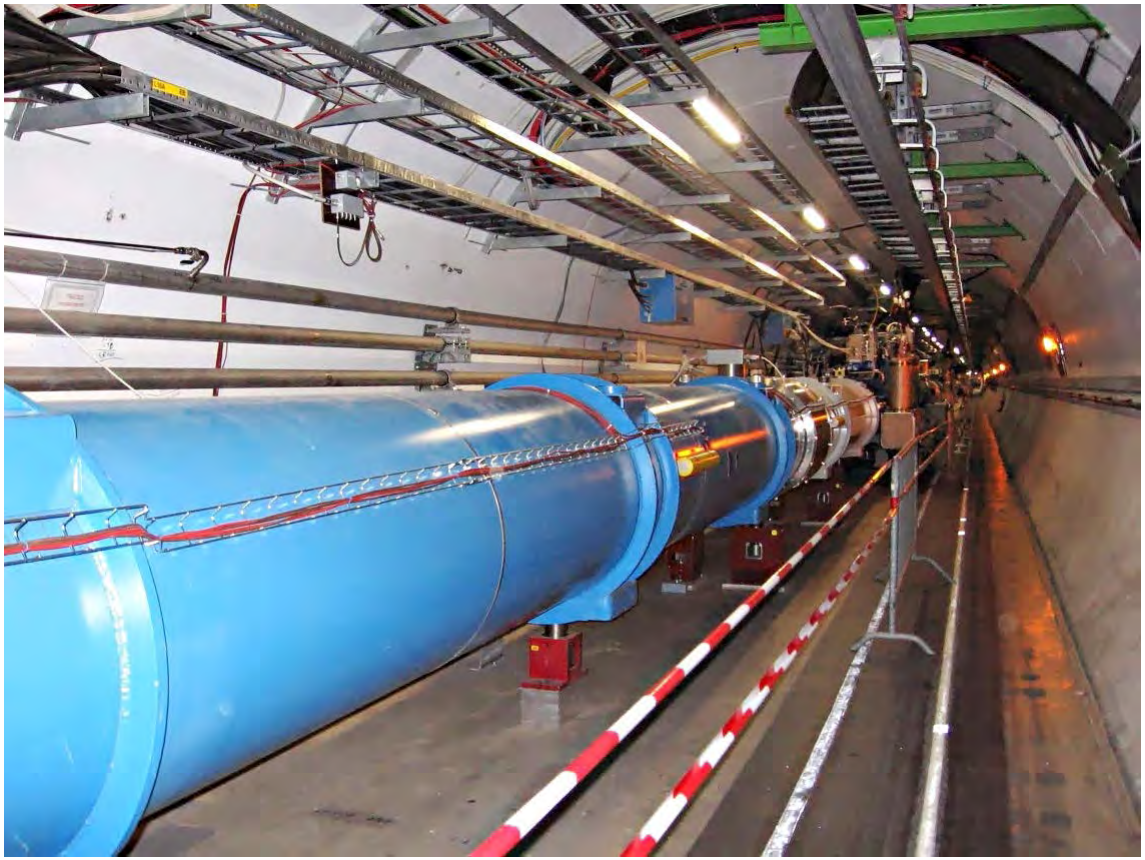
- Accept that predators will eat “anything” within reason and jaw gape.
- Then the challenge is to determine if additional predation is ***sufficient*** to alter population dynamics of the non-target

A suggestion for predators

- Determine the practical prey range, based on
  - size of prey,
  - ability to escape,
  - Innate attractiveness
  - Class of prey (especially intra-guild prey)
  - …

- Proceed to population dynamics studies in the native range.
  - Does the predator occur with the non-target?
  - Does the predator limit population growth/performance of non-targets in the native range?

The apparatus and design will depend on the organism



The apparatus and design will depend on the organism BUT:

- Scientific review will comment on the capacity of the organism to perform in the test conditions

Considerations

- Age and experience of organisms should be standardized
- Conditions (biotic and abiotic) should be suitable for oviposition/development
- Organisms should be associated with normal host plants and other cues
- The designs should be properly replicated

Testing development: no-choice designs – for example, number completing development in groups of 10 attacks

Host	Group (each with 10 females)	Yes	No
Target	1	8,5,9,…	2,5,1,…
Target	2		
Target	3		
NT1	1		
NT1	2		
NT1	3		
NT2	1		
NT2	2		
NT2	3		

An example data table

30 available parasitoids, each provided 10 target, or one of two non-target hosts

host	group	replicate	Offspring	No offspring
target	1	1	3	7
target	1	2	6	4
target	1	3	8	2
target	1	4	9	1
target	1	5	2	8
target	1	6	8	2
target	1	7	5	5
target	1	8	7	3
target	1	9	9	1
target	1	10	9	1
nontarget	1	1	2	8
nontarget	1	2	4	6
nontarget	1	3	5	5
nontarget	1	4	1	9
nontarget	1	5	6	4
nontarget	1	6	4	6
nontarget	1	7	2	8
nontarget	1	8	3	7
nontarget	1	9	7	3
nontarget	1	10	2	8
And so on…				

data summary from ten groups of ten attacks for each female

Host	N	Mean proportion developing to adult ± SE
Target	10	0.78 ± 0.039
NT1	10	0.36 ± 0.062
NT2	10	0.19 ± 0.046

replication

Variation (standard error)

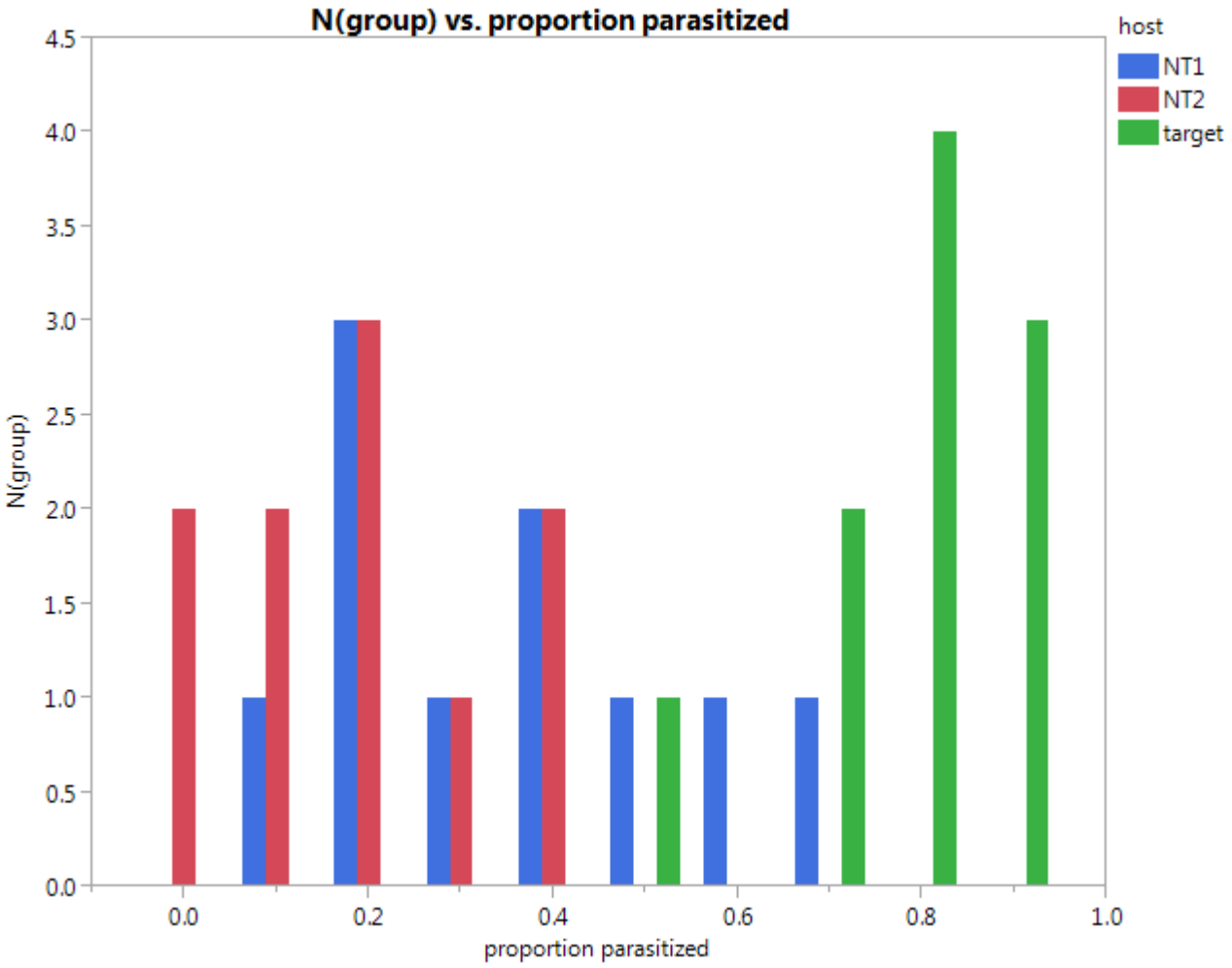
Statistical tests: Least-Squares model (assumes normal (Gaussian) distribution)

Host	N	Mean proportion developing to adult ± SE
Target	10	0.78 ± 0.039a
NT1	10	0.36 ± 0.062b
NT2	10	0.19 ± 0.046b

Analysis of Variance

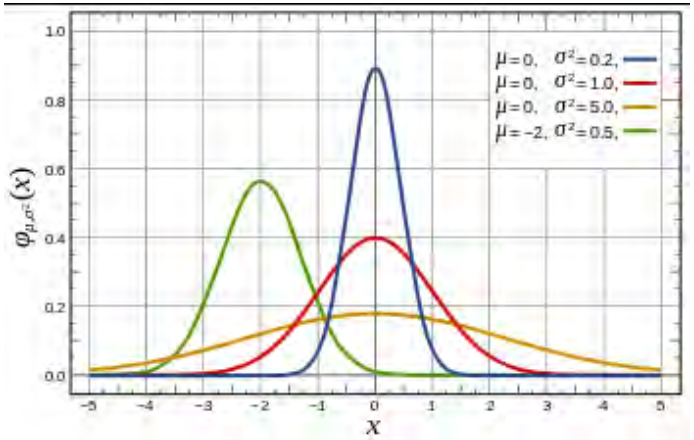
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.8446667	0.922333	37.2242
Error	27	0.6690000	0.024778	Prob > F
C. Total	29	2.5136667		<.0001*

Testing for normality

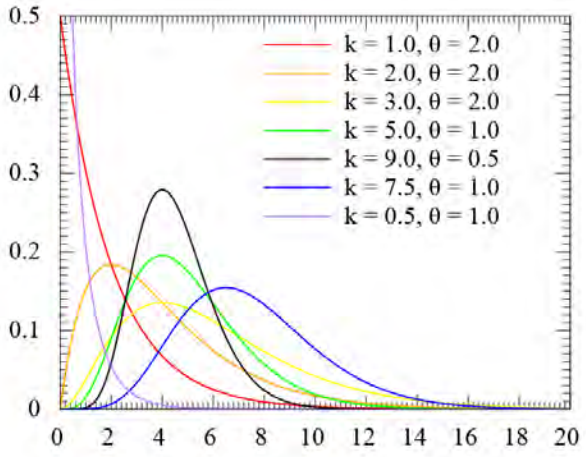


Data distributions are important

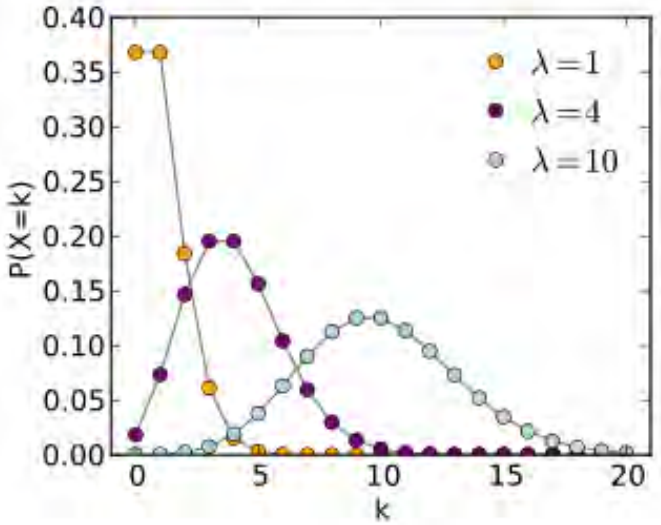
Gaussian – continuous



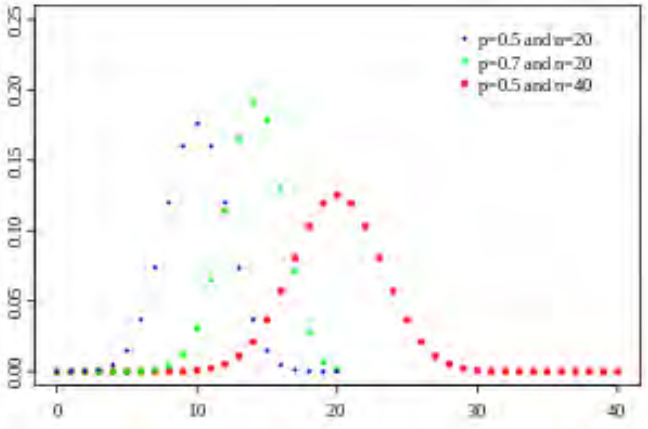
Gamma - continuous



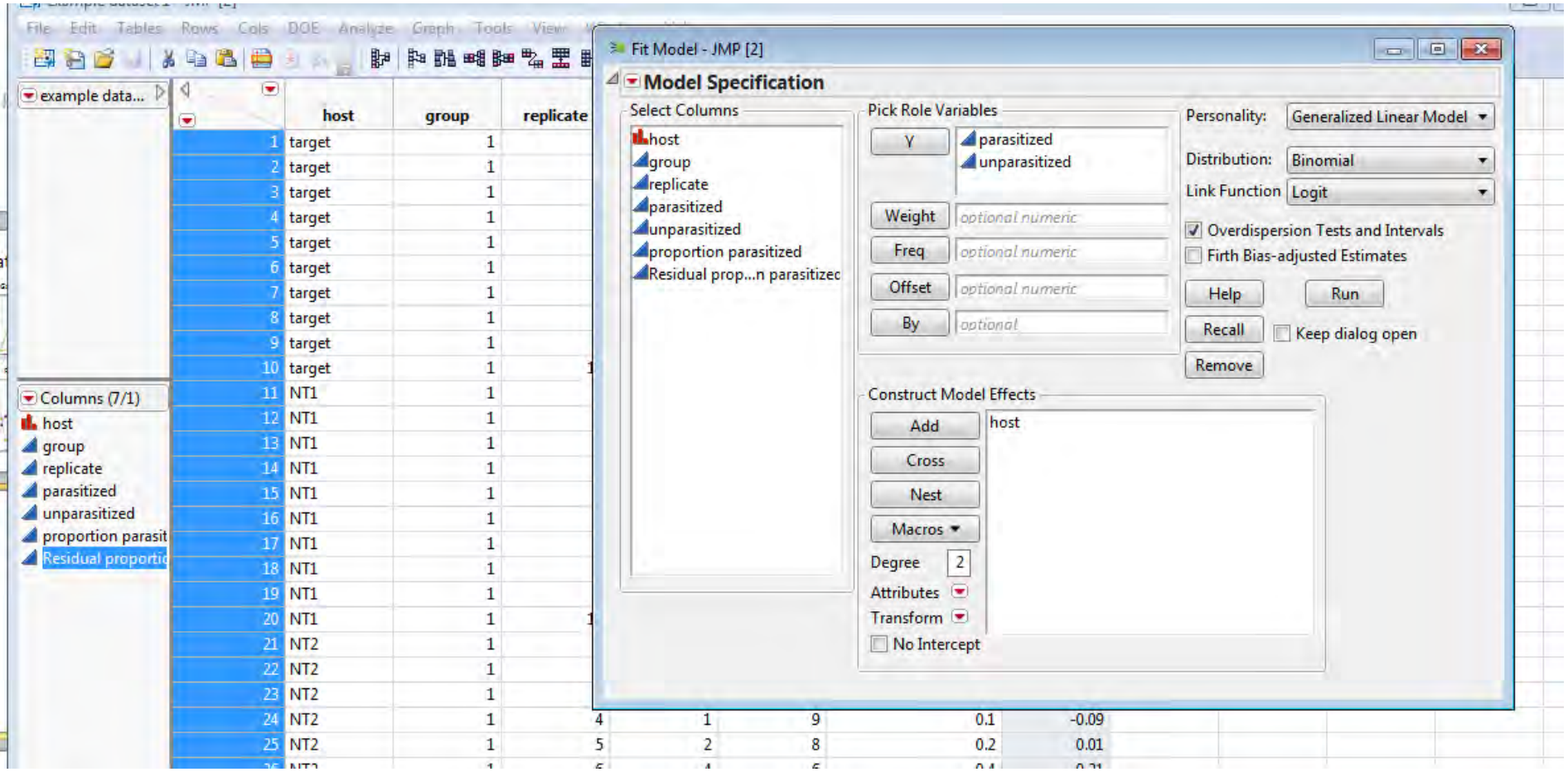
Poisson - count



Binomial – yes/no

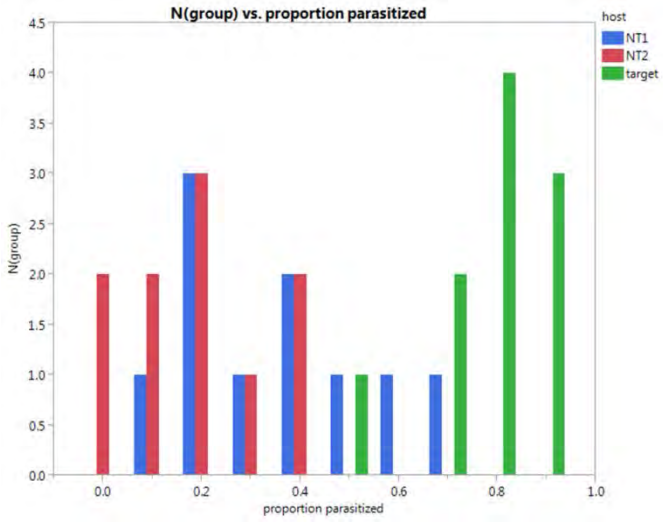


Generalized Linear Models



Generalized Linear Models (JMP)

- Binomial distribution
- Overdispersion
- Target and NT1 are not different
- Target and NT2 are different



Generalized Linear Model Fit

Overdispersion parameter estimated by Pearson Chisq/DF
Response: parasitized
Distribution: Binomial
Link: Logit
Estimation Method: Maximum Likelihood
Observations (or Sum Wgts) = 19

Whole Model Test

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	3.35923392	6.7185	2	0.0348*
Full	15.9971572			
Reduced	19.3563911			

Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq	Overdispersion
Pearson	38.0440	16	0.0015*	2.3777
Deviance	42.6348	16	0.0003*	

AICc
42.8515

Effect Tests

Source	DF	L-R ChiSquare	Prob>ChiSq
host	2	6.7184678	0.0348*

Parameter Estimates

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	5.3010008	1433.72	0.7520831	0.3858	-0.70526	12.088193
host[NT1]	-5.691867	1433.72	1.7268571	0.1888	-12.55853	0.4294168
host[NT2]	-6.483696	1433.72	6.681885	0.0097*	-13.23293	-0.372108

Generalized linear models Binomial Distribution

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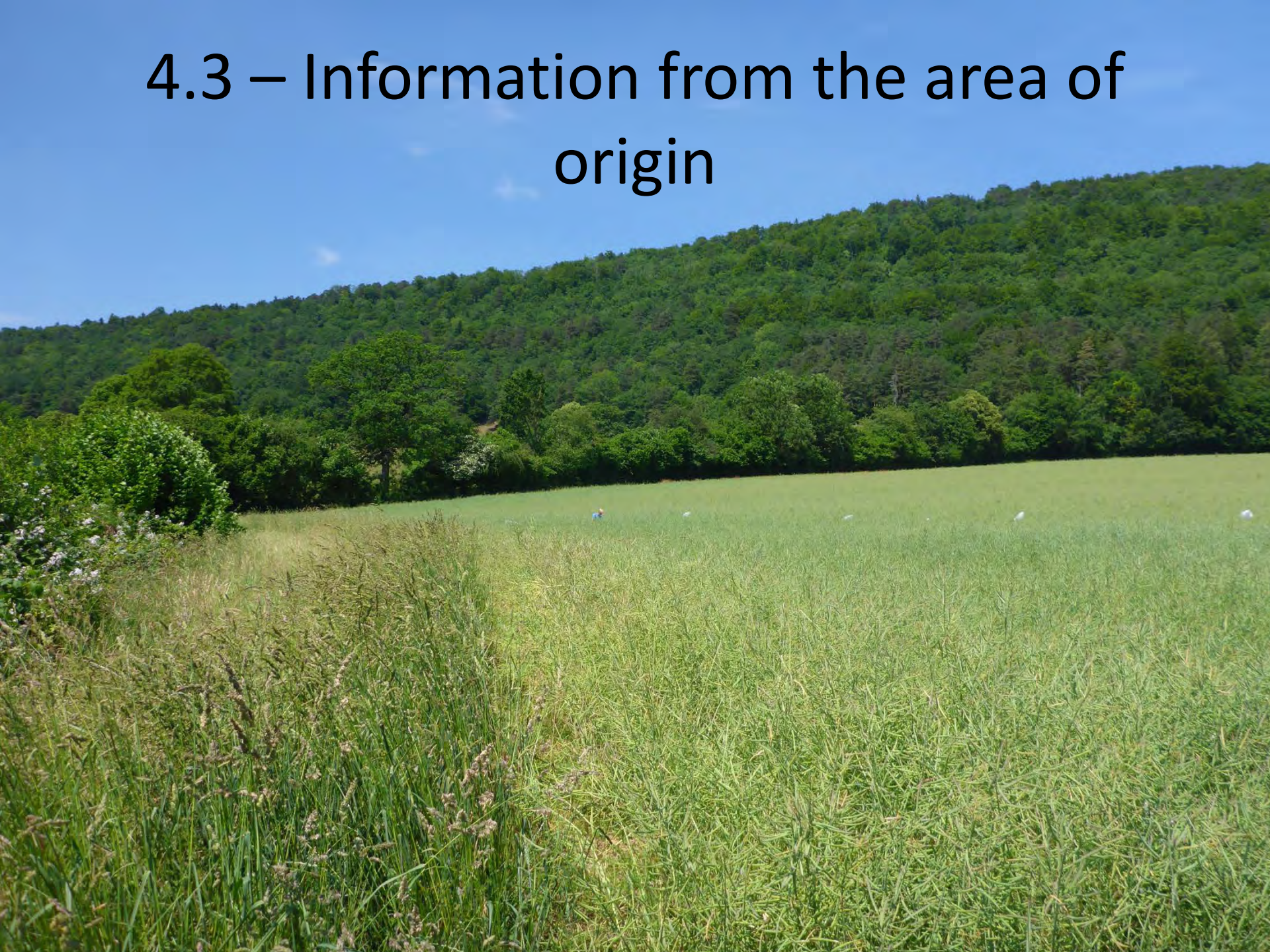
How should these results be interpreted?

A caveat:

- Although host specificity testing in the laboratory can provide a lot of useful information, these experiments do not define the “realized” host (or prey) range

- The **realized host (or prey) range** is determined by interactions with seasonal timing, distribution, dispersal, habitat, climate, other organisms…

4.3 – Information from the area of origin



4.3 – Information from the area of origin

1. Information from scientific literature, natural history museums
   - Are there records from related/similar hosts or prey?
   - Museum/literature records are incomplete!

4.3 – Information from the area of origin

2. Surveys to determine incidence in non-target populations
   - Does the agent occur in conjunction with non-target populations? Climate matching to determine most likely distributions

4.3 – Information from the area of origin

3. Field exposures of non-targets in the presence of agent populations
   - Determine if attack occurs at a rate that generates concern
   - What is an acceptable level of mortality?

.

4.3 – Information from the area of origin

4. Field cage (greenhouse?) tests with agent and non-targets

   - These might be relevant in some cases. Standards of design are similar to those for laboratory studies.

Some experimental design pitfalls:

- Use the appropriate data distribution
  - Determines the ability to detect an effect.
- Replication
  - Determines the ability to detect an effect
- Independence
  - Affects the validity of experimental designs
- Pseudoreplication
  - Often unavoidable in host range tests
- Type I errors
  - This error is additive for related experiments
  - Bonferroni corrections may be needed
- Type II errors
  - The power of the test determines the confidence with which you accept the null hypothesis

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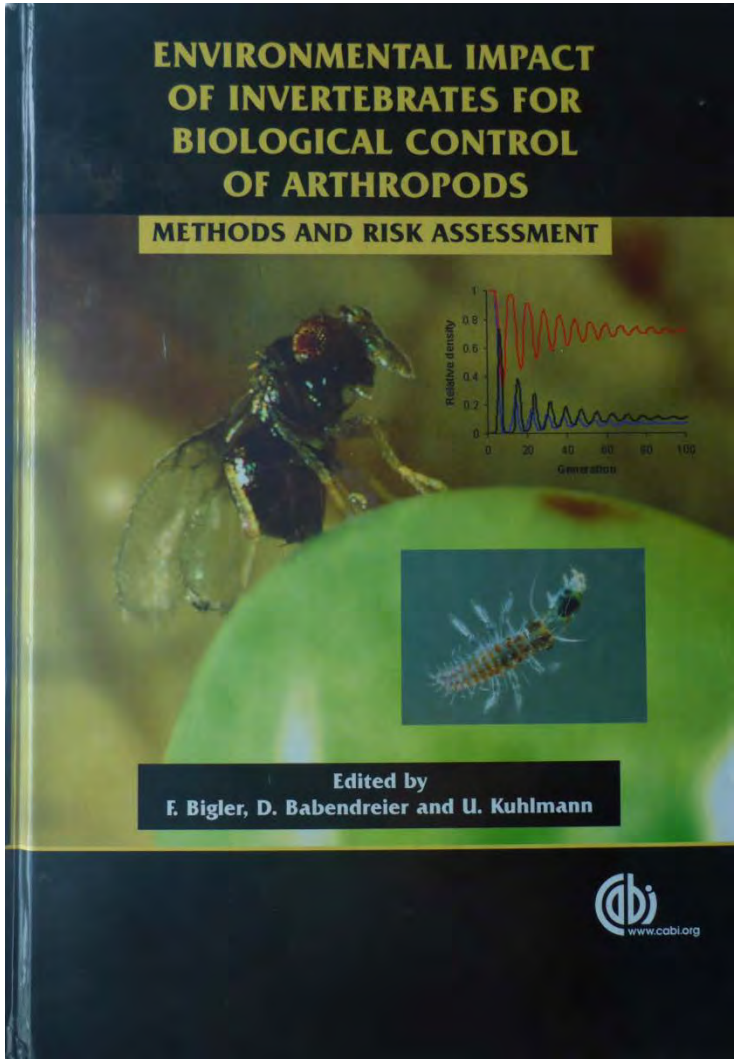

Summary

- Host specificity testing is not simple
- Tests must be consistent with standard principles of experimental design
- Data analysis must be appropriate to the data
- Reports must be complete and describe all aspects of the test(s)
- Results of host specificity testing should inform the discussion of impacts in section 5


Discussion?