

Proof of Safety for Non-target Species

A Confidence Interval based Approach

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Agricultural Field Trials - Today and Tomorrow - 2007

Gefördert durch: Bundesministerium für Bildung und Forschung,
Forschungsvorhaben 0313269 - Biologische Sicherheit gentechnisch veränderter Pflanzen.

Objective

We want to compare the abundance of insect species between conventional crops and genetically modified cultivars

- Provide evidence, that a novel treatment does NOT lead to relevant changes compared to a standard treatment
- Assumption of Poisson or negative binomial distribution for insect counts

Here, we want to investigate

- Coverage probabilities of confidence interval methods for the ratio of mean abundances
- Dependency between sample size, safety margins, and the parameters of the assumed distribution

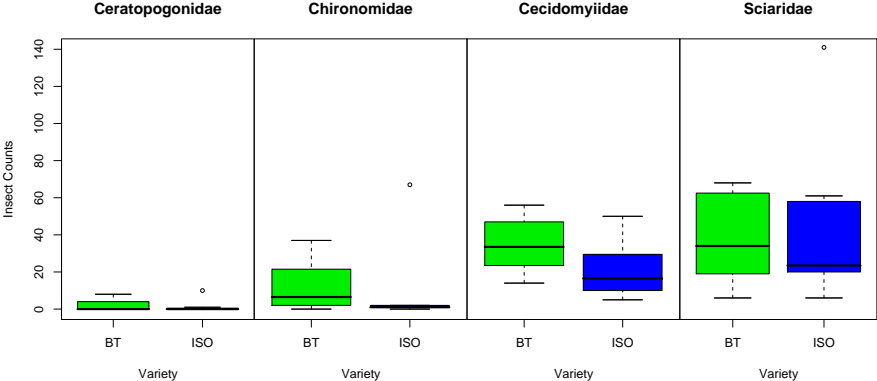
Example

As an example, we present data of an ecological field trial, comparing insect abundance between an isogenic and a Bt-maize variety

- Randomized complete block design with 8 blocks
- Data collected by soil eklektor traps in summer 2005
- Here only data for animals of the suborder Nematocera in the order Diptera
 - ▶ Ceratopogonidae (biting midges)
 - ▶ Chironomidae (non-biting midges)
 - ▶ Cecidomyiidae (gall midges)
 - ▶ Sciaridae (fungus gnats)

Data provided by S. Prescher, Biologische Bundesanstalt Braunschweig, Germany

Example



Proof of Safety

m_1 : mean of standard; m_2 : mean of novel treatment;
 $r_l < r_u$: safety margins

$$H_0^L : \frac{m_2}{m_1} \leq r_l \quad \text{OR} \quad H_0^U : \frac{m_2}{m_1} \geq r_u$$

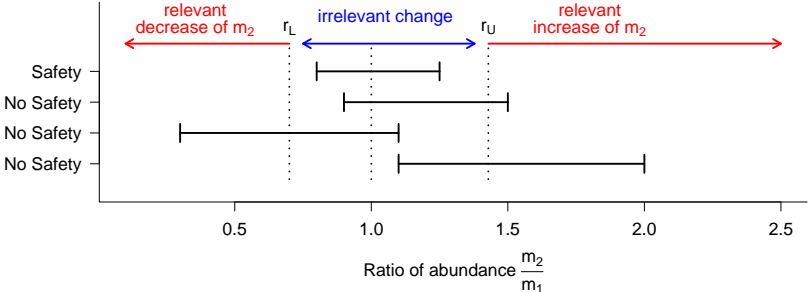
$$H_A^L : \frac{m_2}{m_1} > r_l \quad \text{AND} \quad H_A^U : \frac{m_2}{m_1} < r_u$$

Safety can be declared, if BOTH Null-Hypotheses are rejected
(each at level $\alpha = 0.05$)

Alternatively, $(1 - 2\alpha)$ confidence intervals can be constructed
Safety can be declared, if the lower AND upper confidence bound are
BOTH included between the safety margins

Proof of Safety

Conclusion for safety only if the $(1 - 2\alpha)$ -confidence interval is included in safety margins $[r_L, r_U]$



Distribution of Insect Counts

Assumption of Poisson distribution

- Positive integers
- Variance completely determined by the mean

Negative Binomial distribution

- Accounting for extra variation
- Poisson distribution as a special case

Fixed Effects Model

2-Sample Comparisons

We can set up the fixed effect model

$$\log(m_i) = a_i$$

where a_i is the treatment effect with $i = 1, 2$

Generalized Linear Model

Estimation is done in a generalized linear model framework with a log-link under assumption of

$$\text{Poisson distribution} \quad V(X) = m$$

$$\text{Negative Binomial distribution} \quad V(X) = m + \phi m^2$$

The parameter ϕ accounts for extra variation in the observed samples and has to be estimated from the data

As an outcome, we obtain

- Estimates $\hat{a}_2 - \hat{a}_1$ with $\log\left(\frac{\hat{m}_2}{\hat{m}_1}\right) = \hat{a}_2 - \hat{a}_1$
- with standard error $\hat{S}_{a_2 - a_1}$

Approximate Confidence Intervals

2-Sample Comparisons

We construct simple Wald intervals
by inversion of two 1-sided $(1 - \alpha)$ tests

$$\frac{m_2}{m_1} \in \left[\exp \left(\hat{a}_2 - \hat{a}_1 \pm z_{1-\frac{2\alpha}{2}} \sqrt{\widehat{s}_{\hat{a}_2 - \hat{a}_1}^2} \right) \right]$$

Also profile-likelihood intervals or confidence intervals based on resampling might be an alternative, but no clear improvement toward the Wald intervals could be shown by simulation

Trials in several Environments

We can extend the previous model to a **fixed effects model including environmental effects**, like years or locations:

$$\log(m_{ik}) = e_{ik} = a_i + b_k + c_{ik}$$

a_i Treatment effect $i = 1, 2$

b_k Environment effect $k = 1, \dots, K$

c_{ik} Interaction between treatment and environment

Interest might be in the difference of the treatment effects $a_2 - a_1$

or in detecting safety for all environments by k intervals for the differences $e_{2k} - e_{1k}$

Simulation Study

We tried to cover a wide part of the parameter space by varying

- the numbers of observations
- the means abundance in Standard
- the ratio of mean abundance in Novum to Standard
- the distribution of the response

Simulation Study

Software

- Simulations are done with the free available statistics software R
- GLMs fitted with the add-on package gamlss
- Confidence intervals constructed with the add-on package multcomp

2-Sample Comparisons

Parameter settings

- Sample size

$$J = 5, 10, 20, 100$$

- Mean abundance in Standard

$$m_1 = 0.1, 0.5, 1, 5, 10, 50$$

- Ratio of mean abundance in Novum to Standard

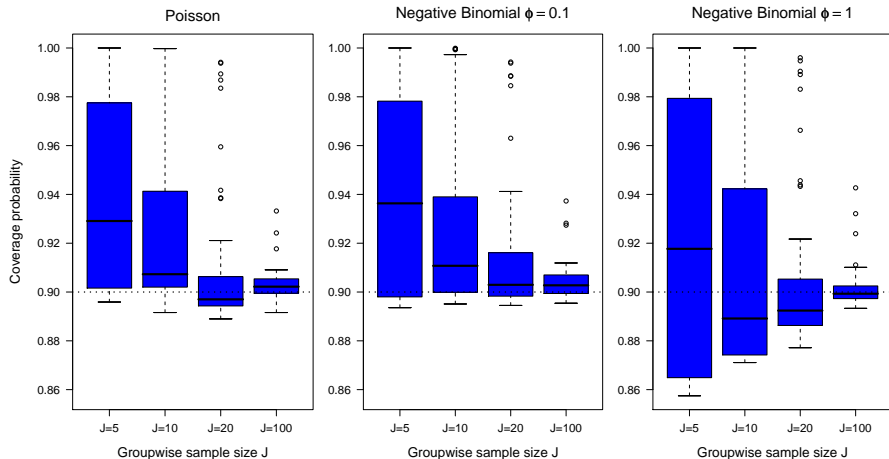
$$\frac{m_2}{m_1} = 0.1, 0.5, 0.8, 1, 1.25, 2, 10$$

- Distribution of the response

- ▶ Poisson(m)
- ▶ negative binomial(m, ϕ), with $\phi = 1, 10$

2-Sample Comparisons

Coverage Probabilities of nominal 0.90 CI



Several Environments

Parameter settings

Number of replications	Number of environments	Total
J	K	JK
8	3	24
4	6	24
2	12	24
10	5	50
5	10	50
2	25	50
20	5	100
10	10	100
5	20	100
2	50	100

Several Environments

Parameter settings

- Mean abundance in Standard

$$m_1 = 0.1, 0.5, 1, 5, 10, 50$$

- Ratio of mean abundance in Novum to Standard

$$\frac{m_2}{m_1} = 0.1, 0.5, 0.8, 1, 1.25, 2, 10$$

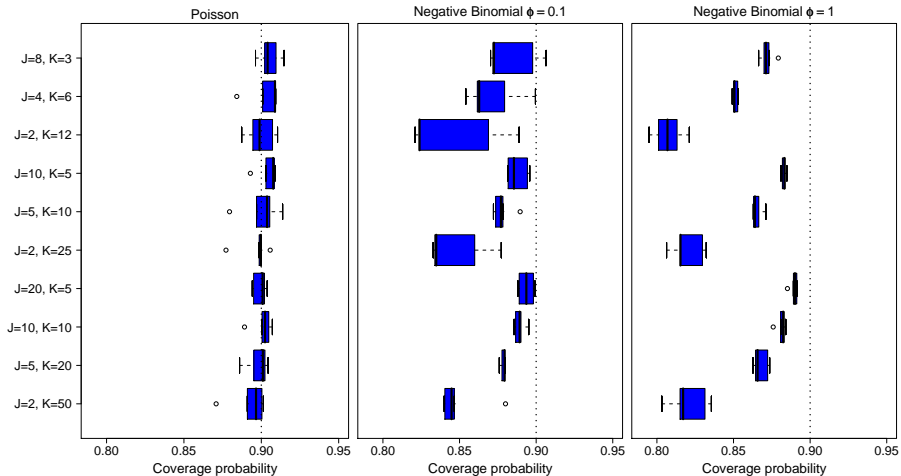
- Distribution of the response

- ▶ Poisson(m)
- ▶ negative binomial(m, ϕ), with $\phi = 1, 10$

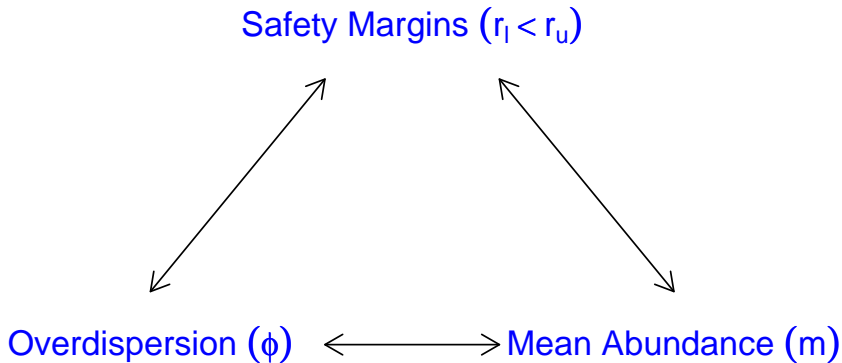
- Means of the environments b_k are sampled from a Normal distribution $N(0, 1)$ on the log-link
- No interaction considered ($c_{ik} = 0$)

Several Environments

Coverage Probabilities of nominal 0.90 CI in a fixed effects model



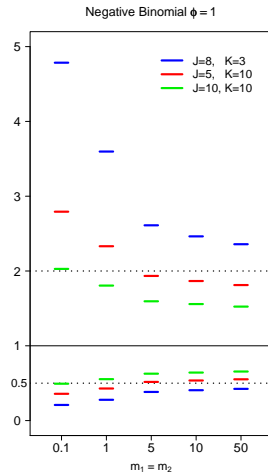
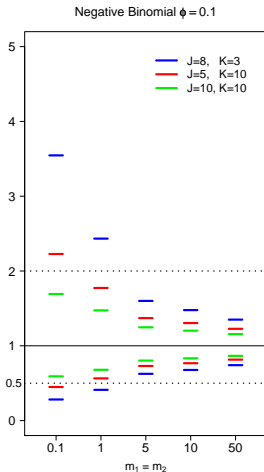
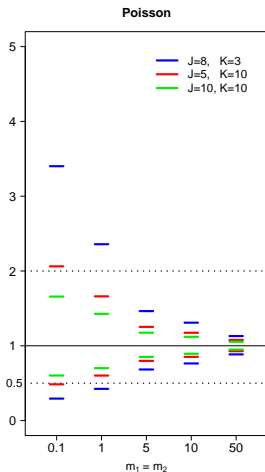
Bermuda Triangle



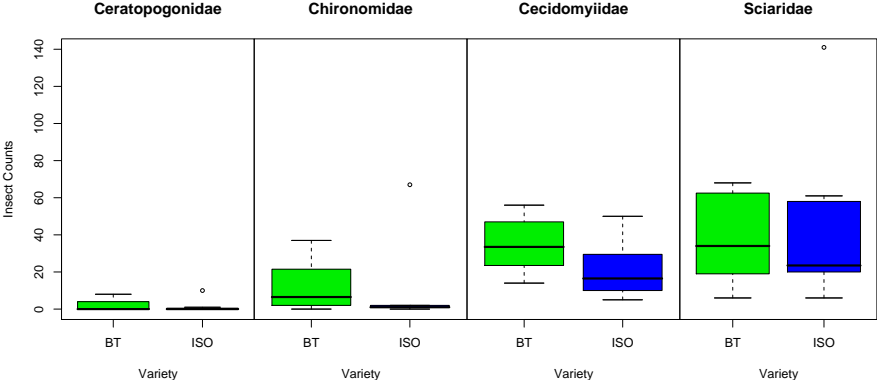
Safety Margins

- Under assumptions of complete equality of mean abundances ($\frac{m_2}{m_1} = 1$), we search for an experimental design in which we can conclude safety with a high probability
- Safety margins are a-priori determined on the basis of ecological criteria
- We are interested in the smallest possible safety margins, where we could reach a power of 0.8

Safety Margins



Example



Example

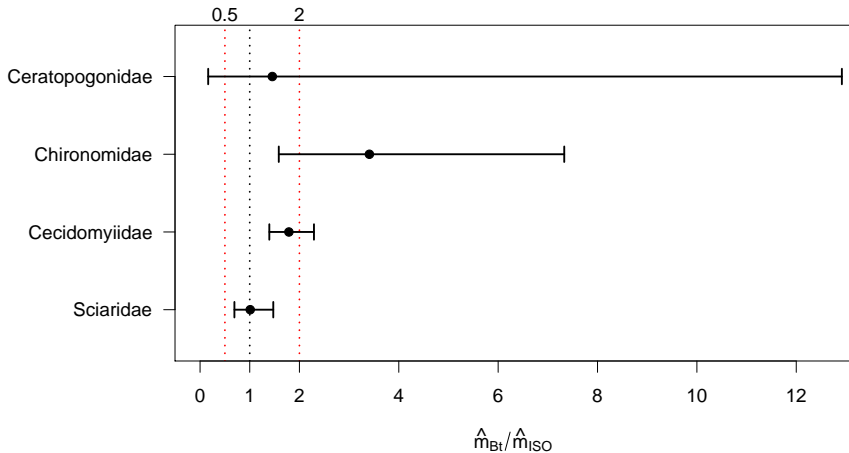
GLM Results

Species	Estimate		Dispersion $\hat{\phi}$	Replicates J
	\hat{m}_{Bt}	\hat{m}_{ISO}		
Ceratopogonidae	2.00	1.38	0.17	8
Chironomidae	3.68	1.08	0.48	8
Cecidomyiidae	38.48	21.52	0.05	8
Sciaridae	13.03	12.91	0.18	8

Example

Proof of Safety

$(1 - 2\alpha)$ confidence intervals for the ratio $\frac{m_{Bt}}{m_{ISO}}$



Dealing with Multiple Species

(See previous talk)

Until now, we are only dealing with single species, but often several species are considered

- Summarize species according to ecological criteria
 - ▶ Beneficial especially for rare species with low mean abundance
- A global safety decision may be not sufficient
 - ▶ Identifying a subset of species which are safe (Quan 2001 [7])
 - ▶ Adjusting for multiplicity will make proof of safety even harder

Recommendations and further Research

- Proof of Safety is hardly possible for
 - ▶ rare species (mean abundance < 5)
 - ▶ occurrence of high overdispersion ($\phi > 0.1$)
 - ▶ small sample sizes ($J < 20$)
- Confidence intervals may violate nominal level at high overdispersion

Outlook:

- Extension to complex designs by use of generalized linear mixed models
- Dealing with multiple species
- Pooling taxonomic groups by means of identical characteristics may be a way to handle rare species
- Improve confidence intervals, for example by Bayesian methods

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Safety Margins

Distribution	m_1, m_2	$J = 8, K = 3$		$J = 5, K = 10$		$J = 10, K = 10$	
		Lower margin	Upper margin	Lower margin	Upper margin	Lower margin	Upper margin
Poisson	0.5	0.294	3.401	0.485	2.062	0.603	1.658
	1	0.424	2.358	0.602	1.661	0.701	1.427
	5	0.683	1.464	0.798	1.253	0.851	1.175
	10	0.764	1.309	0.852	1.174	0.893	1.120
	50	0.885	1.130	0.928	1.078	0.949	1.054
Negative Binomial $\phi = 0.1$	0.5	0.282	3.546	0.449	2.227	0.591	1.692
	1	0.411	2.433	0.564	1.773	0.679	1.473
	5	0.625	1.600	0.730	1.37	0.802	1.247
	10	0.677	1.477	0.767	1.304	0.832	1.202
	50	0.741	1.350	0.815	1.227	0.865	1.156
Negative Binomial $\phi = 1$	0.5	0.209	4.785	0.358	2.793	0.493	2.028
	1	0.278	3.597	0.429	2.331	0.554	1.805
	5	0.383	2.611	0.517	1.934	0.627	1.595
	10	0.406	2.463	0.536	1.866	0.642	1.558
	50	0.424	2.358	0.552	1.812	0.656	1.524