

# Design of experiments and analysis of data concerning GM crops

Joe Perry  
Visiting Worker:  
Plant & Invertebrate Ecology Division  
Rothamsted Research



Peter Rothery  
CEH Monks Wood



Suzanne Clark  
Biomathematics & Bioinformatics Division  
Rothamsted Research



# Farm Scale Evaluations of GMHT Crops

Three experiments

Spring-sown crops:

Beet, Spring oilseed rape (SOSR), Maize  
all GMHT

1999 (pilot year for protocols)

1999 power study

experiments begun in 2000

power study reported February 2003

Results reported October 2003

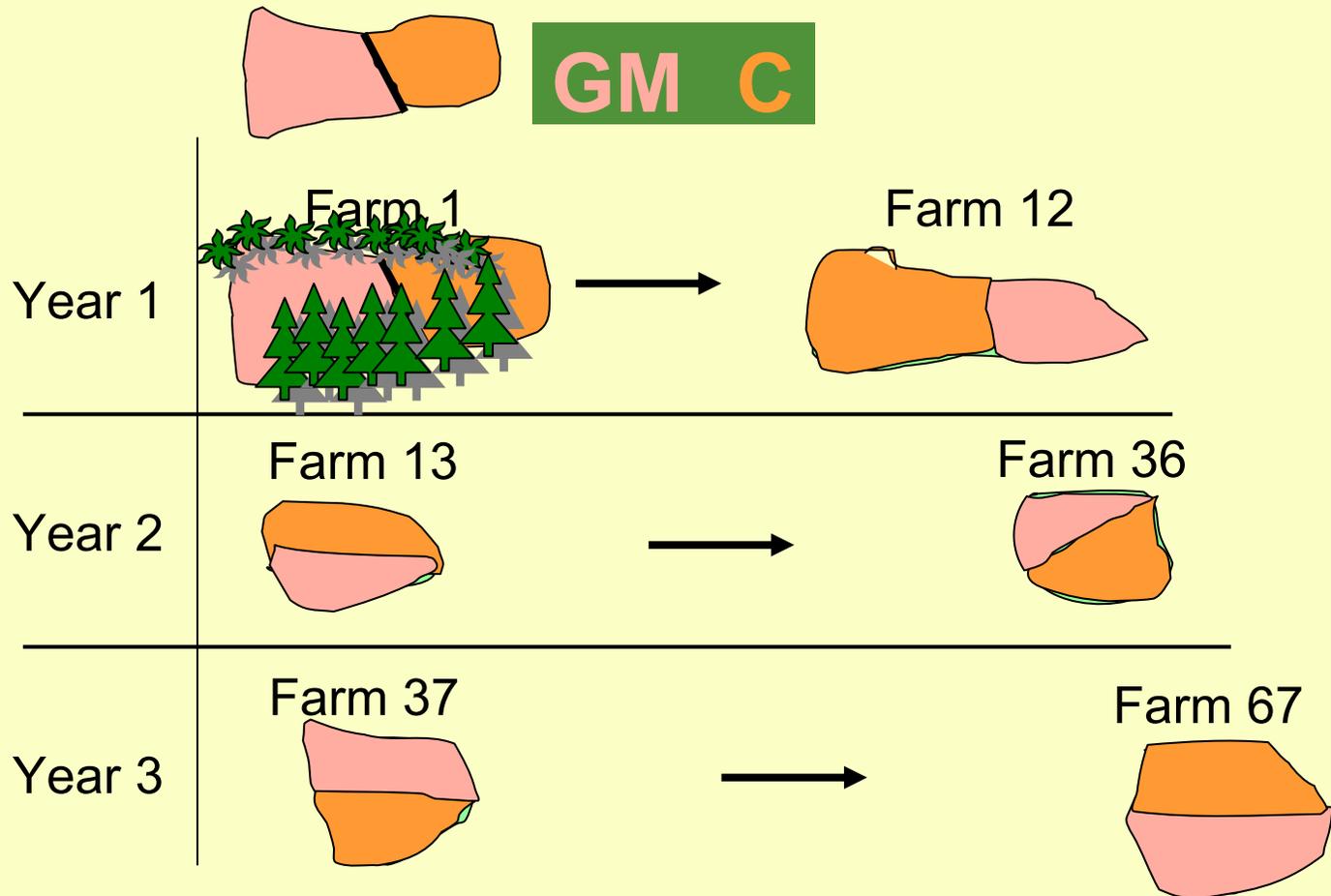
# The null hypothesis: $H_0$

- There is no difference between the management of GMHT varieties and that of comparable conventional varieties, in their effect on the abundance and diversity of arable plants and invertebrates.
- Wildlife – not food safety or gene flow



# Statistical design of experiment

Randomized block: experimental units are half-fields



Perry et al. (2003) *Journal of Applied Ecology*, **40**, 17–31

# Power Simulation Study

## Model

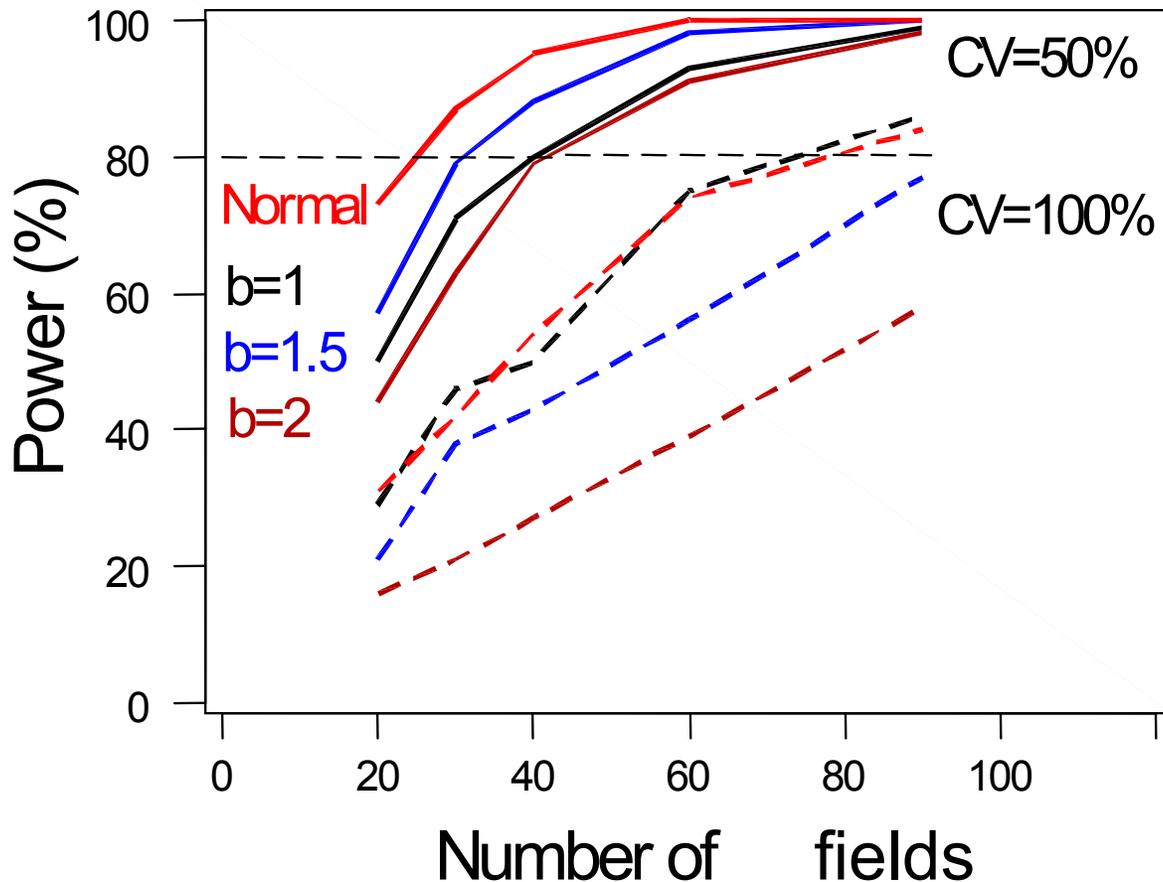
- mean  $\alpha$  treatment effect (R) x crop effect x field effect
- count = negative binomial distribution  $1/k = (V^2 - m) / m^2$
- variance-mean relationship: power law -  $V = am^b$   
( $b = 1, 1.5, 2$ ). For negative binomial we have  $k = m^2 / (am^{b-1} - 1)$
- variability measured through CV%, where for negative binomial  $CV^2 = 1/m + 1/k$  and we studied CV% = 50%, 80%, 100%.
- field effects covered each of four ranges of means:

mean	Range
(1)	0.1 - 10
(5)	0.5 - 50
(10)	1.0 - 100
(50)	5.0 - 500

- power estimated from 500 sets of simulated data, with  $P$  values computed from paired Monte Carlo randomization test

# Statistical Power

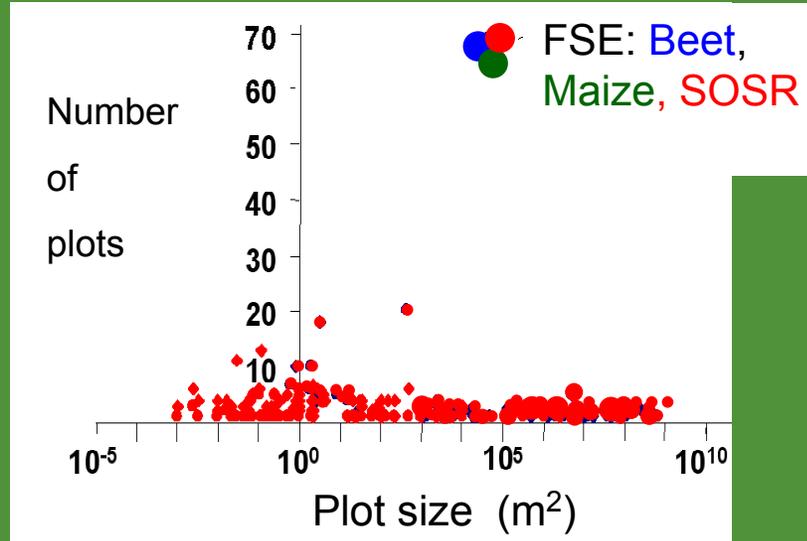
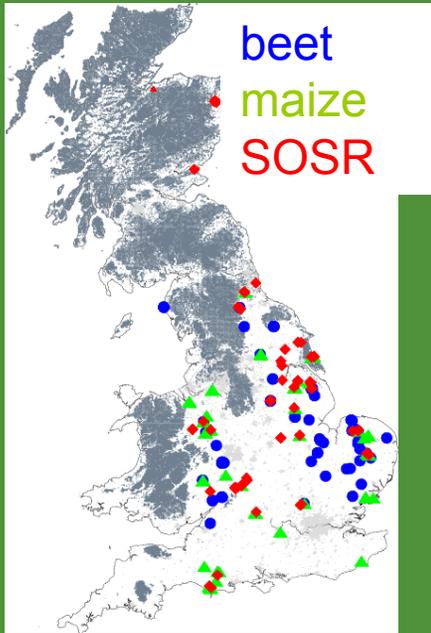
assumed treatment effect,  $R$ ,  
represents 1.5-fold difference



# Design considerations

- Halved-fields or paired whole fields?
- GMHT management = cost-effective weed control
- Sites – representative of where crop grown in GB
- Fields – representative of size

# Size of the FSE



The size of the FSE versus 82 comparable ecological experiments

“This research will not only address GM crops. This is an extremely important opportunity to gain a more detailed understanding of the effects of agricultural management on farmland wildlife generally”

*Michael Meacher, 1999*

We counted: 700,000 plants; 17,000 bees; 13,000 butterflies

We trapped: 500,000 seeds; 1.5 million invertebrates

We made >4000 visits to fields; analysed >7000 datasets

# Analysis estimation

$d$  = mean difference in log-transformed catch between GM and C

$$\begin{aligned} \text{in this example, } d &= -2.32 + 2.34 \\ &= 0.02 \end{aligned}$$

Site	C	GM
1	2.571	2.467
2	3.201	3.444
3	0	1
4	1.10	2.53
↓	↓	↓
66	0	0
67	31	42
67	1.49	1.62

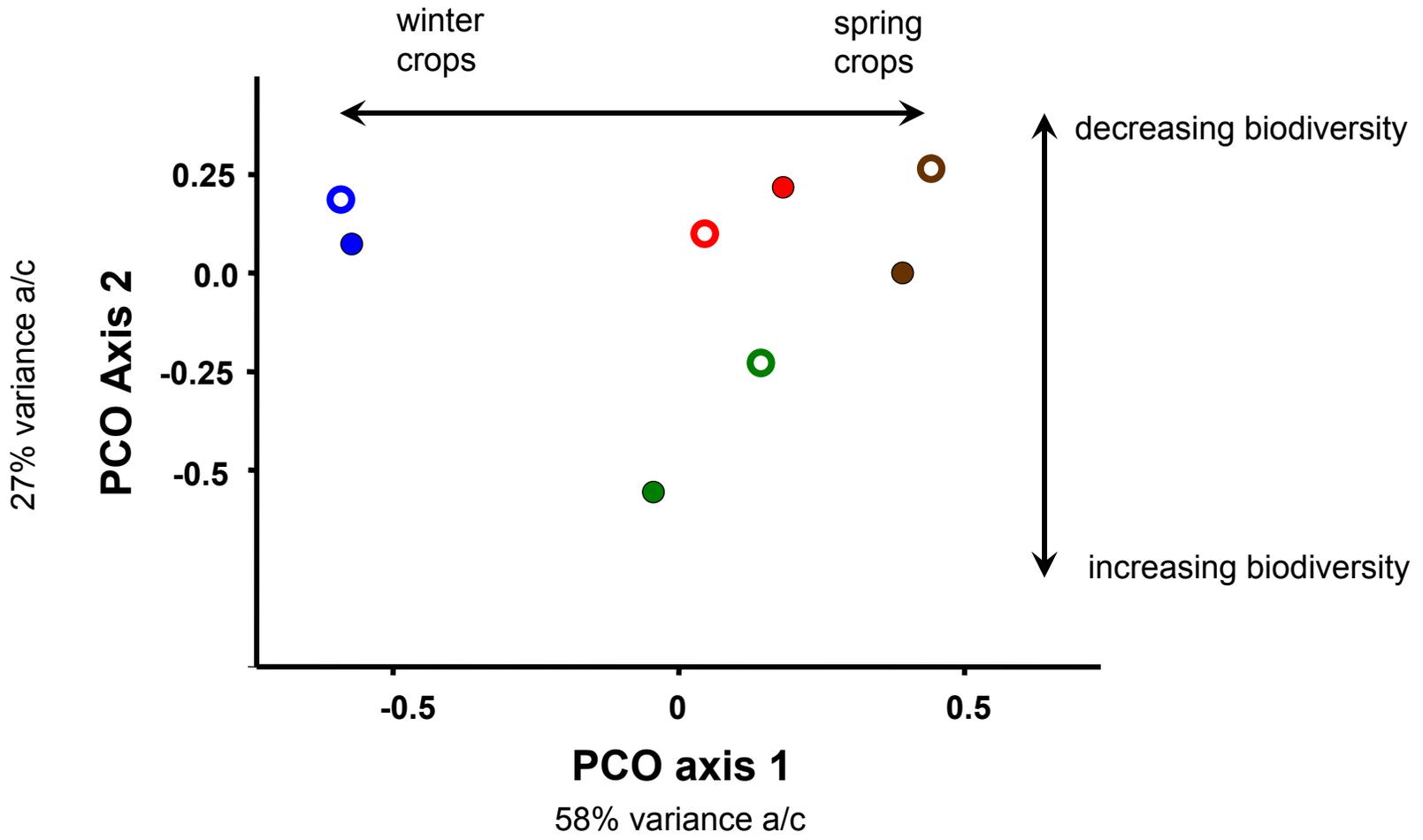
A value of  $d = 0.02$  on the transformed scale is equivalent to  $d = 0.02 \times \sqrt{V}$ . Assume  $V \propto \mu^2$ ,

$N$ ,  $h_0$   $H_0$  tested by randomization test. Involves swapping pairs of values at random within sites, and recomputing  $d$

# Conclusions

- GM cropping **WILL** affect farmland wildlife
- Effects result from differences in herbicide management systems
- Maize beneficial, beet and spring oilseed rape harmful, compared with current conventional
- All depends on management of crop, field & landscape
- Differences between crops *greater* than differences between GM and conventional

<i>Treatment</i>	<i>Crop</i>
● Conventional	SOSR
○ GMHT	WOSR
	Maize
	Beet



[http://www.rothamsted.bbsrc.ac.uk/pie/joe\\_general\\_work\\_GM\\_FSE\\_page\\_3\\_1.htm](http://www.rothamsted.bbsrc.ac.uk/pie/joe_general_work_GM_FSE_page_3_1.htm)



## Joe Perry's work in GM research - The UK Farm Scale Evaluations of GMHT crops



### The role of ACRE in interpreting the results: November 2003 - January 2004

The regulatory body charged with advising Ministers on risk assessment of GM crops is the Government-appointed Advisory Committee on Releases to the Environment ([ACRE](#)). It was ACRE's responsibility to interpret to Ministers the published results of the FSE within a regulatory framework consistent with EU law, as set out in the directive 2001/18/EC. Prior to the release of the FSE results ACRE announced that it would give any interested parties the opportunity to consider the FSE results and their implications and to submit evidence as part of their deliberation process. Evidence was accepted for a period of a month following publication of the results. Sixty eight submissions were received and were made publicly available on the website: <http://www.livegroup.co.uk/acrefarmscaleevaluations>. ACRE held two public open

**Click to download the two methods papers**, published in the *Journal of Applied Ecology* in February 2003.

**The protocols and methods used** from Les Firbank and colleagues:

[Firbank, L.G.](#), Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A., Champion, G., Scott, R., Hill, M.O., Dewar, A., Squire, G.R., May, M., Brooks, D.R., Bohan, D., Daniels, R.E., Osborne, J.L., Roy, D. & Black, H.I.J., Rothery, P. & Perry, J.N., (2003) An introduction to the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology*, **40**, 2-16.



**The design, analysis and statistical power** from Joe Perry and colleagues:

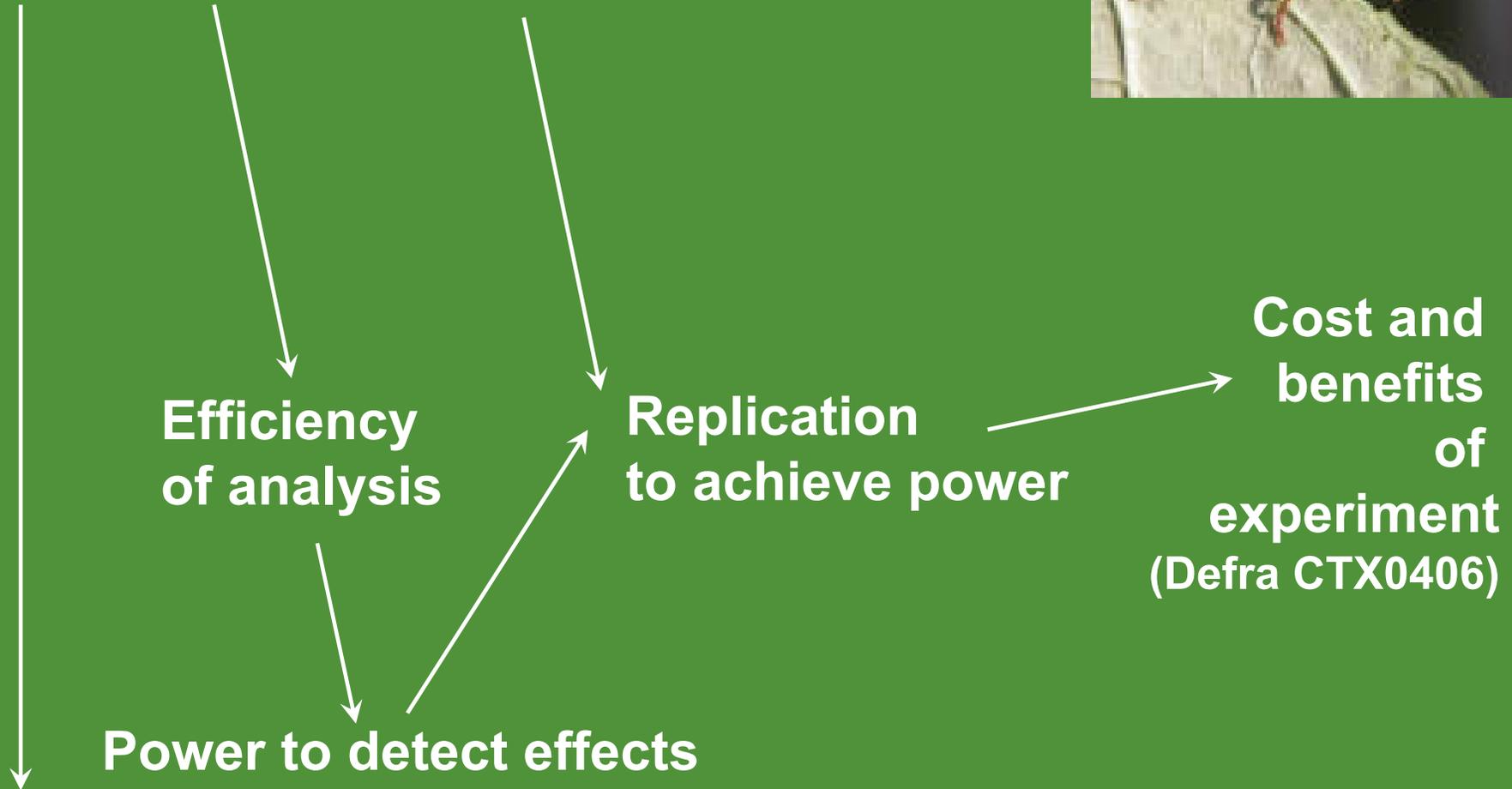
[Perry, J.N.](#), Rothery, P., Clark, S.J., Heard, M.S. & Hawes, C. (2003) Design, analysis and power of the Farm Scale



Downloads include: eight data papers published in *Phil. Trans. Roy. Soc.* (2003), **358**, 1779 - 1913 the Commentary on the FSE (Firbank *et al.*, 2003), and the non-technical summary (Burke *et al.*, 2003)

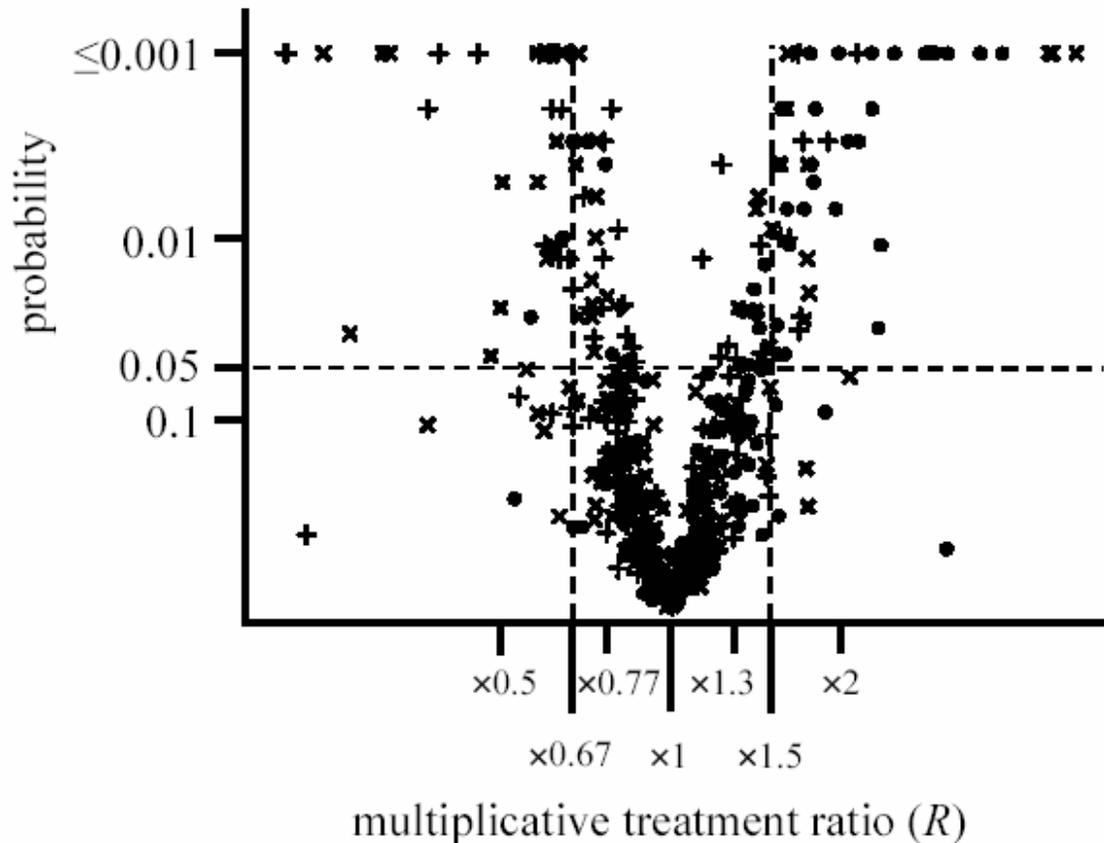
Farm Scale Evaluations of GMHT Crops

$\beta = 1.51$  rate of increase in variability with abundance  
 $CV\% 62.7$  variability per half-field (natural scale)  
 $s = 0.63$  variability per half-field (logarithmic scale)  
 $M = 37.86$  abundance per half-field, as geometric mean



$$\text{Probit [Power]} = 0.955 + 0.670\theta - 0.526\theta \sqrt{M} - 1.46\beta + 0.182\theta\beta - 7.67 \sqrt{n} + 3.62\beta \sqrt{n}$$

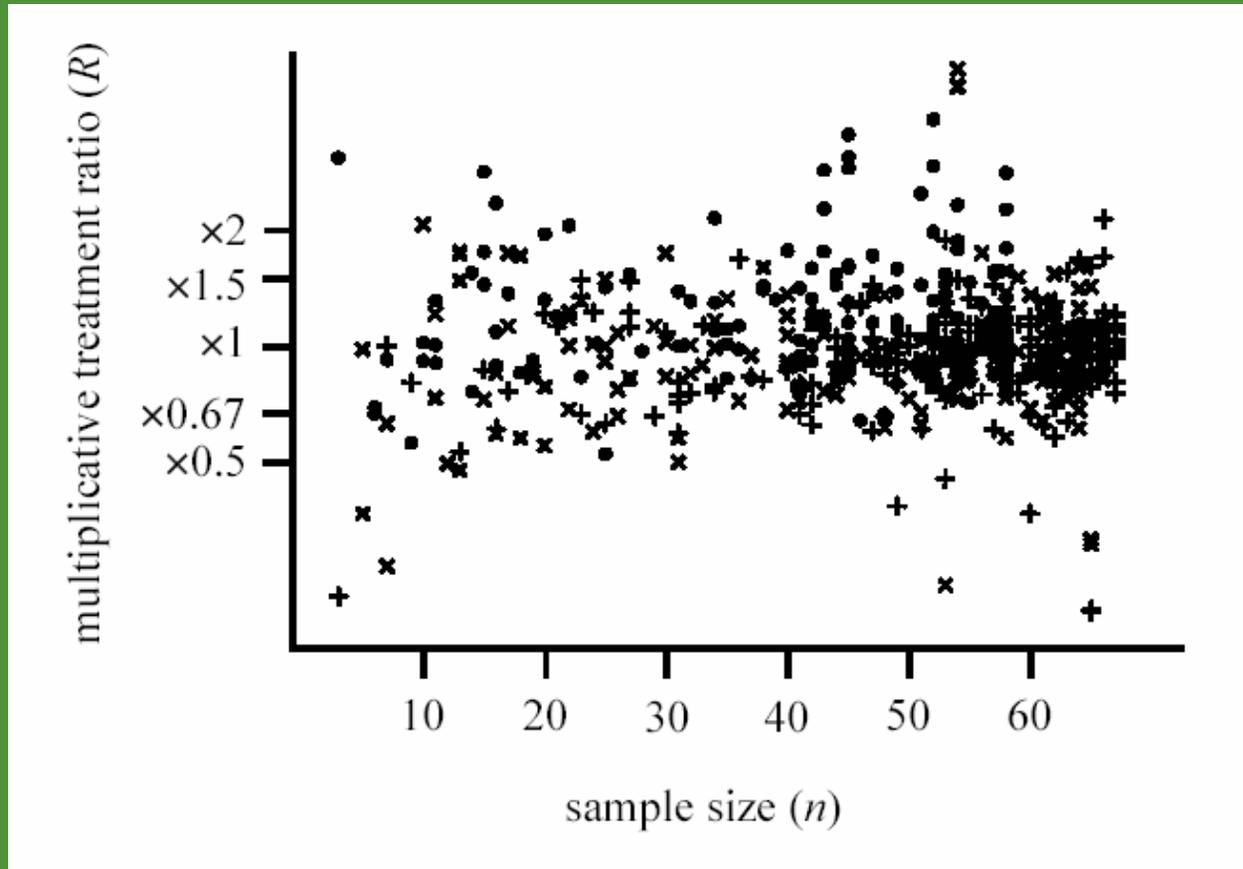
# Detectability of treatment effects (i)



Farm Scale Evaluations of  
spring-sown genetically  
modified herbicide-tolerant  
crops: a statistical  
assessment  
Suzanne J. Clark,  
Peter Rothery and  
Joe N. Perry (2005)  
*Proc. R. Soc. B.*, **273**,  
237 – 243.

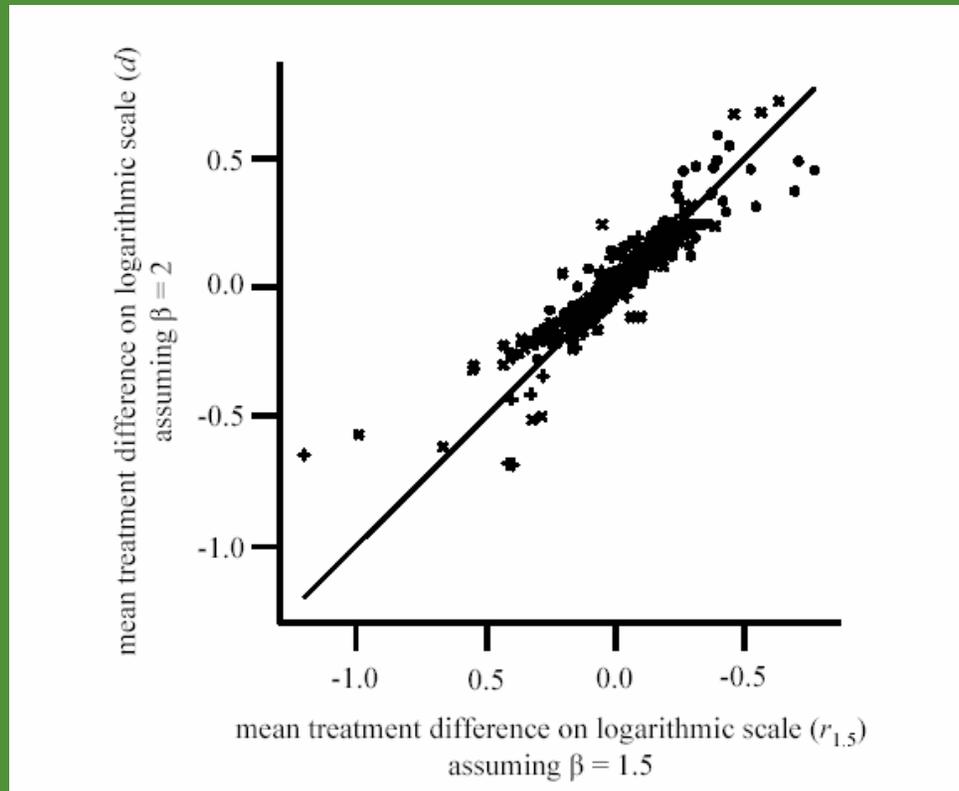
For 110 of the 531 variates  $R$  was  $> 1.5$  or  $< 0.67$  (symbols outside two vertical lines)  
82% of these (those above the horizontal line) achieved significance at the 5% level  
Halving of sample size would result in a loss of about half the significant results

# Detectability of treatment effects (ii)



No relationship between size of treatment effect and realized sample size (i.e. abundance) for any of the three crops

# Checking model assumptions

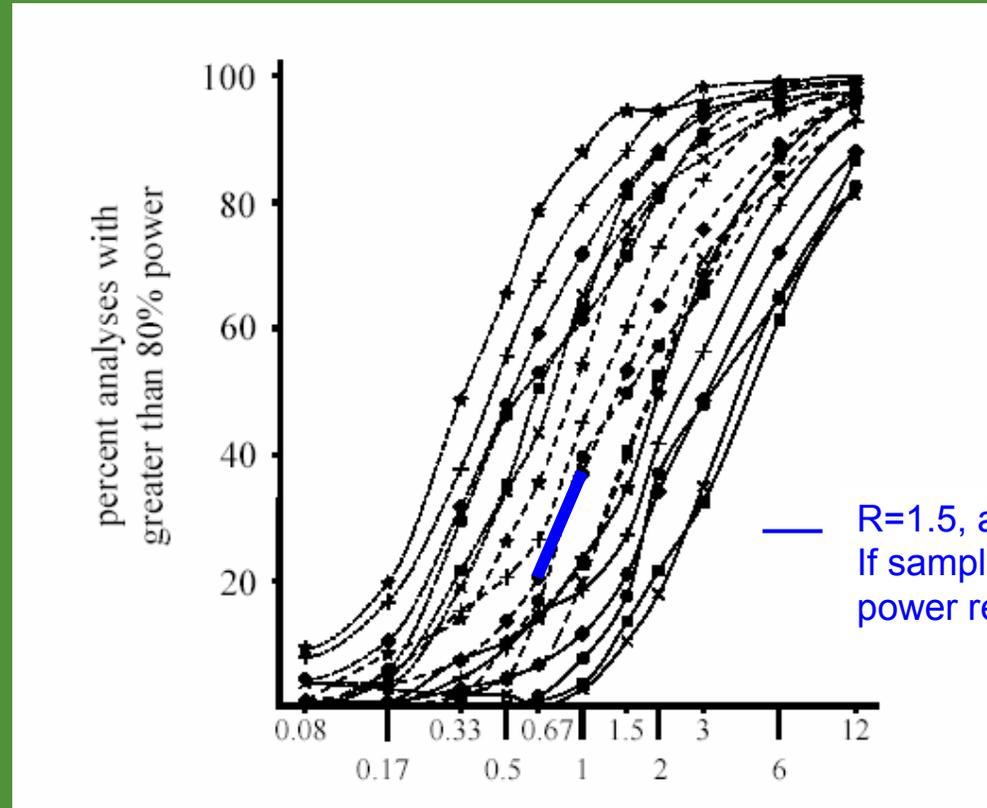


Median values of  $\beta_0$  were consistent between major taxa and between crops; all fell between 1.5 and 2.0, with mean = 1.7.

Inferences appeared robust to model misspecification; in only 4% of cases would 5% significance under one model have given non-significance using the other.

# Effects of change of sample size

$$\text{Probit [Power]} = 0.955 + 0.670\theta - 0.526\theta / \sqrt{M} - 1.46\beta + 0.182\theta\beta - 7.67 / \sqrt{n} + 3.62\beta / \sqrt{n}$$



Fraction of actual achieved sample size, in terms of field replication

# analyses with power  $\geq$  80%

# analyses with power  $\geq$  90%

$R = 1.3$     $R = 1.5$     $R = 2$

$R = 1.3$     $R = 1.5$     $R = 2$

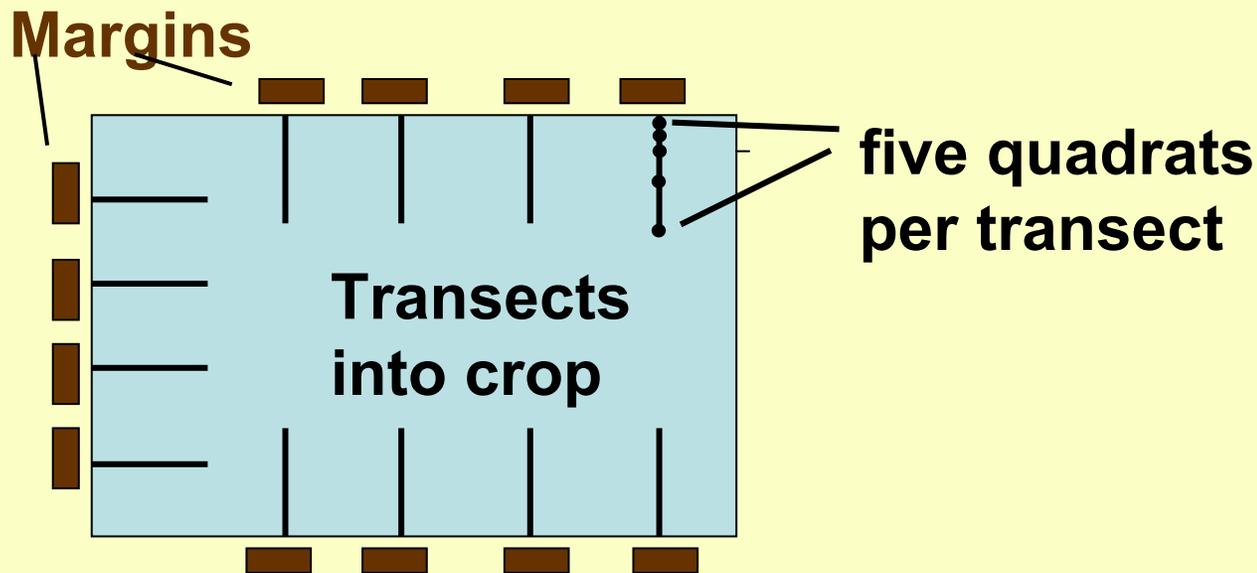
69   211   394

43   147   351

all indicators  
( $N = 531$ )

# Sampling within half-fields

Clark, S.J., Rothery, P., Perry, J.N. & Heard, M.S. (2007)  
Farm Scale Evaluations of herbicide-tolerant crops: assessment of within-field  
variation and sampling methodology for arable weeds.  
*Weed Research*, **47**, 157–163.



Firbank et al. (2003) *Journal of Applied Ecology*, **40**, 2–16

# Effect on $P$ -values of reduction in within-unit sampling intensity (number of transects)

Number of Transects		Percent analyses with :		
		$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.001$
12		76.7	73.3	50.0
6		78.3	68.3	46.7
3		78.3	60.0	45.8

The amount of within-field sampling could have been reduced from 12 transects (60 quadrats) to 6 transects (30 quadrats) or even to 3 transects (15 quadrats), with little reduction in the frequency of detection of treatment effects or of power.

# Costs of the Farm Scale Evaluations

Qi, Perry, Pidgeon, Haylock & Brooks (2008)  
*Annals of Applied Biology* (submitted)

Total cost = c. £5.9m (c. Euro 10m) = 24, 3-year post-doctoral research grants

The mean overall cost per site = £19,453 (c. Euro 33,000) at 2002 rates

Laboratory time was almost 2.5× field work time

Most costly protocol: soil-surface invertebrates, requiring identification to species

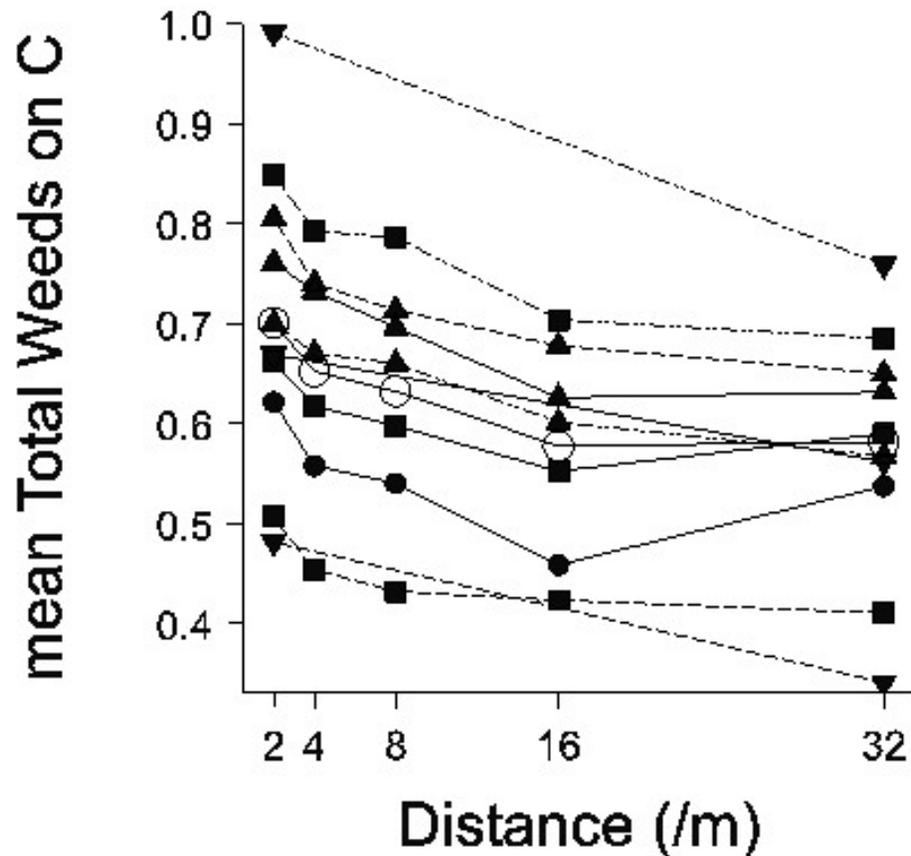
The 'bees and butterflies' identification cost only £418 per site (c. 710 Euro)

The six vegetation protocols 65% of total costs - six arthropod protocols 29%

The recommended reduction from 12 to 3 transects would have saved £1,356 per site, only 6% of total budget.

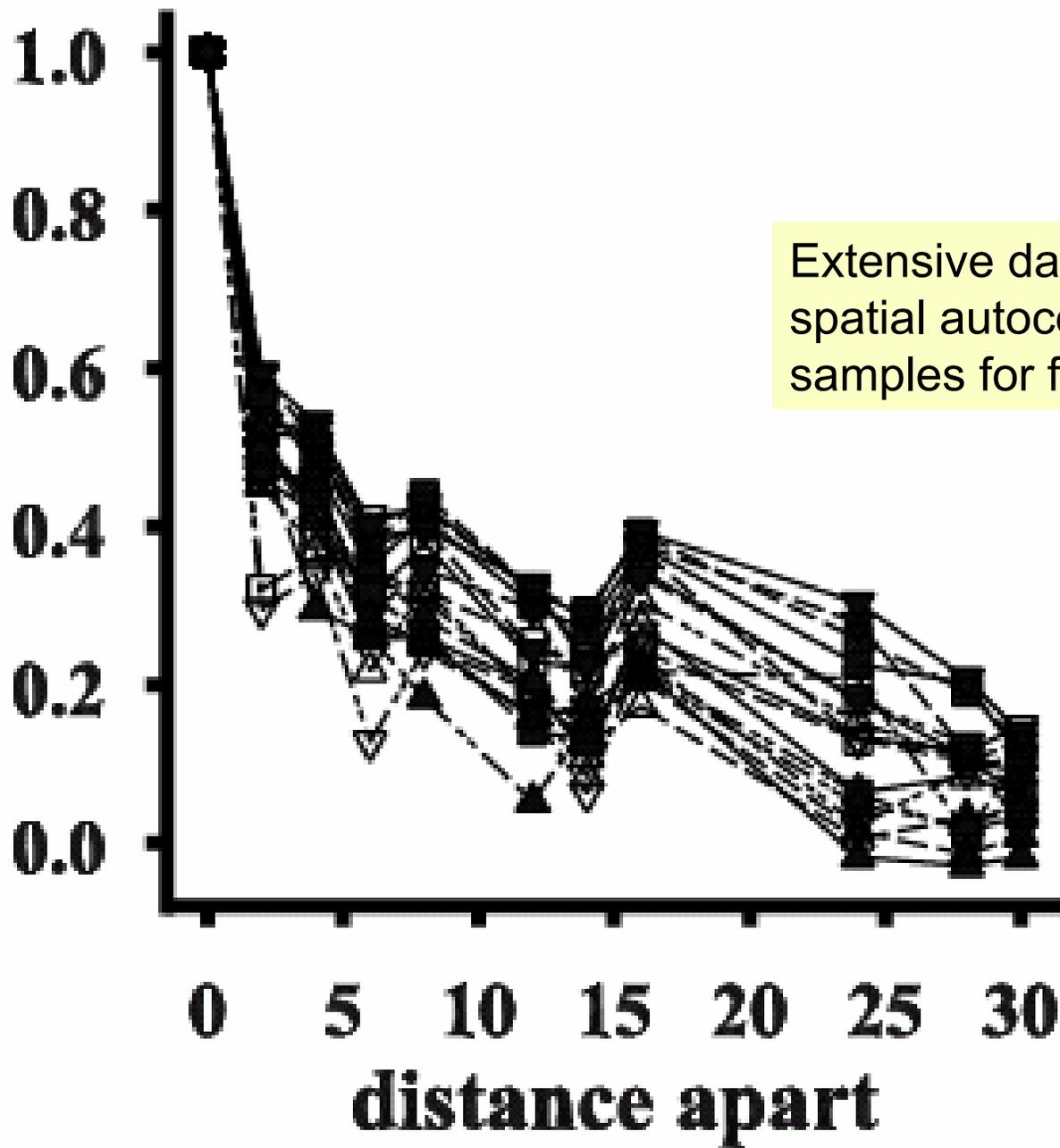
A minimalist approach using only the single season seedbank protocol would have cost only £3,437 (c. 5850 Euro) per site

The effect of geographic spread of sites on cost was small



Extensive data helps to quantify the well-known observation of a decline of weeds from field margins into centre of crop

**correlation**



Extensive data helps to quantify the spatial autocorrelation between samples for farmland weeds



# Predicting longer-term changes in weed populations under GMHT management

Matt Heard, Peter Rothery Joe Perry, Les Firbank

*Weed Research* (2005), 45, 331-338.

**model longer-term population changes over 30 years**

**nine broad-leaved weed species**

**four-year crop rotations**

**conventional cereals, GMHT beet & GMHT rape**

**Seedbanks = weed seed populations in the soil**



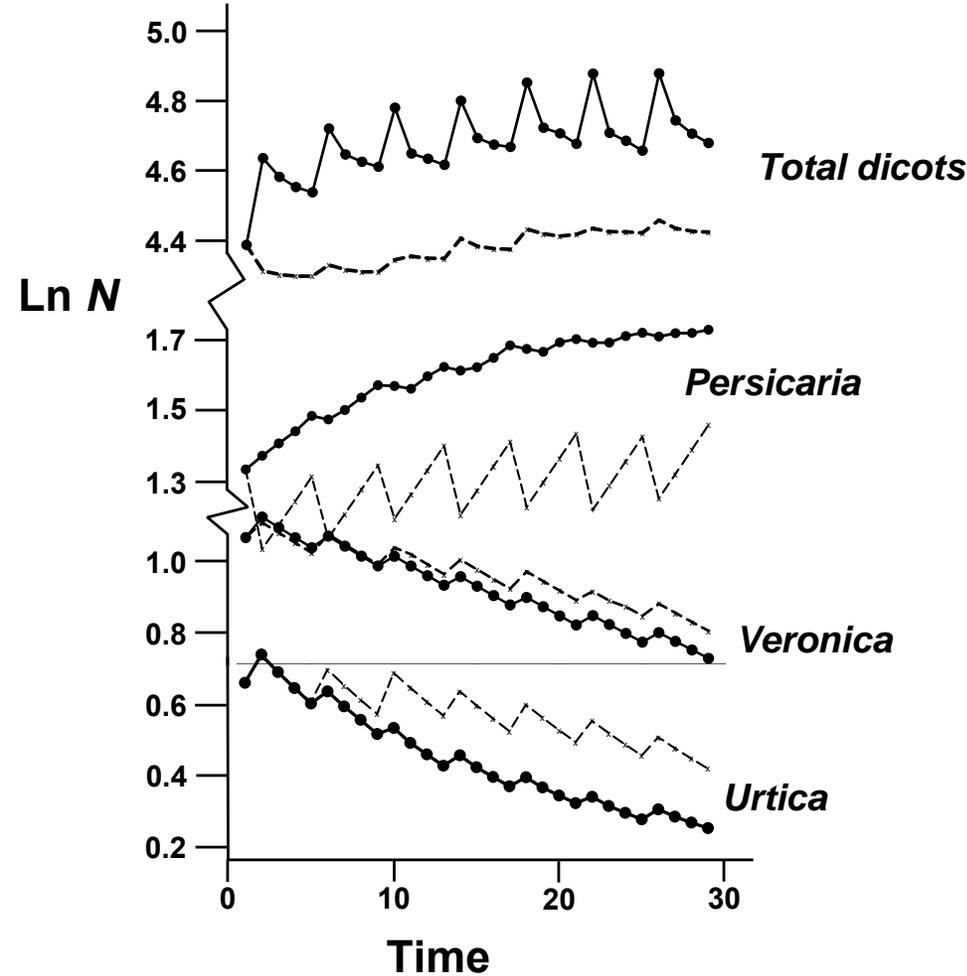
**Conventional**



**GM**



Used density-dependent mathematical models to predict weed densities in break crop / cereal rotations. The density-dependence includes population dynamics & management



Rotation type	Species	Trend		Treatment ratio, R (GM:Conventional)		
		C	GM	Rotation #1 years 1-4	Rotation #3 years 9-12	Rotation #7 years 25-28
Beet, Cereal x 3	<i>Capsella bursa-pastoris</i>	-	-	0.90	0.83	0.82
	<i>Chenopodium album</i>	-	+	0.88	1.01	1.68

# Mitigation in GMHT beet



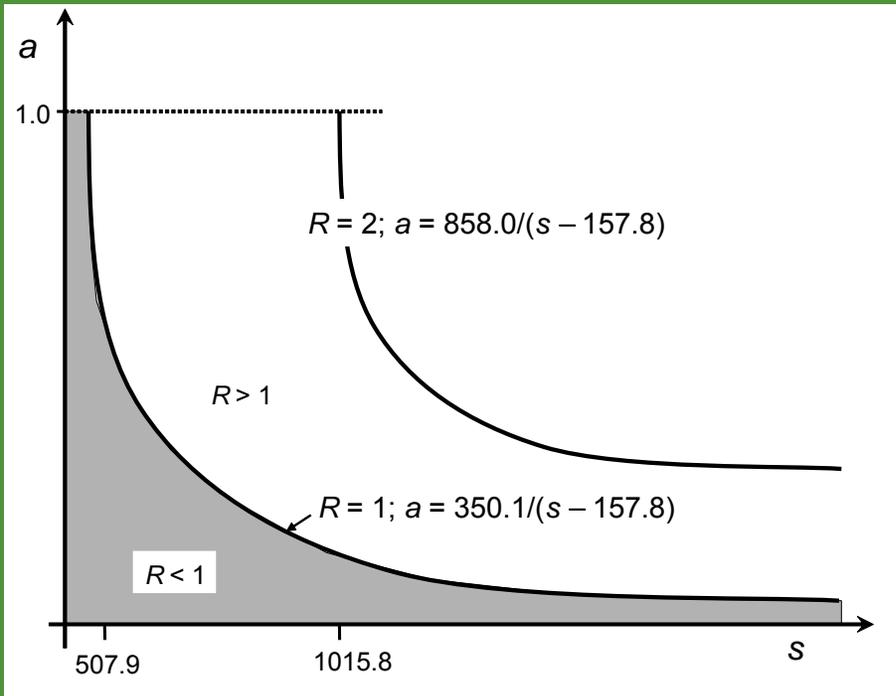
Broom's Barn Research Station



Pidgeon, J.D., May, M.J., Perry, J.N. & Poppy, G.M. (2007)  
Mitigation of indirect environmental effects of GM crops.  
*Proceedings of the Royal Society series B.*  
Published online 18 April 2007; doi:10.1098/rspb.2007.0401.

$s_{\max}$  total dicot seed rain in untreated area ( $\text{m}^{-2} \text{y}^{-1}$ )  
 $a$  proportion of rows of field untreated  
 $1-a$  proportion receiving GMHT management as practiced in the FSE.  
 $R$  ratio dicot seed rain mitigated GMHT : Conventional management.  
 $C(s)$  estimated seed rain, conventional management:  $507.9 \text{ m}^{-2}$   
 $GM(s)$  estimated seed rain, GMHT management:  $157.8 \text{ m}^{-2}$   
 $R_o$  estimated multiplicative treatment effect =  
 $C(s) / GM(s) = 0.31$  with 95% CI of (0.19, 0.53)

$$R = [a(s_{\max} - GM(s)) + GM(s)] / C(s)$$



Require as least as much seed rain in mitigated GMHT as in Conventional ( $R = 1$ ), so minimum value of  $a$  given by:

$$a = [C(s) - GM(s)] / [s_{\max} - GM(s)]$$

Crucial parameter to estimate is  $s_{\max}$ .  
Estimates available from four field trials,  
and by extrapolation from multiple  
regression of data from 65 FSE beet sites

a maximum of 2% of field area left unsprayed is required  
to mitigate weed seed production,  
and 4% to mitigate weed biomass production.