

## Meta-analysis is a powerful tool to summarize variety mixture effects - exemplified by grain yield and weed suppression of spring barley

Lars Kiær<sup>1,2</sup>, Ib Skovgaard<sup>2</sup>, Hanne Østergård<sup>1</sup>

<sup>1</sup> Risø National Laboratory, DK-4000 Roskilde, Denmark, [lars.kiaer@risoe.dk](mailto:lars.kiaer@risoe.dk) and [hanne.oestergaard@risoe.dk](mailto:hanne.oestergaard@risoe.dk)

<sup>2</sup> Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark, [ims@kvl.dk](mailto:ims@kvl.dk)

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### Abstract

In a new project, we aim to increase the general understanding of the power of meta-analysis to combine existing experimental results on variety mixtures. In this way, explanatory power can be increased compared to separate analyses and overall measures and relationships may be revealed. We will thus pursue to uncover a number of critical issues, including the relative importance of various traits and trait combinations on mixing success. To demonstrate the methods of meta-analysis applicable for variety mixture data, we considered a data set consisting of grain yield and weed ground cover assessments in 16 field trials of six 3-component variety mixtures and their components (part of the Danish BAR-OF field trials). The effects of mixing were analysed separately for each field trial and the results used in a meta-analysis in combination with their standard errors. We also analysed the mixing effects of each mixture by fitting a linear model to the entire data set. Both methods showed an overall positive mixing effect on grain yield and a trend for less weed to be found in variety mixtures. Finally, strengths and shortcomings of the methods are highlighted.

### Introduction

In experimental field trials of variety mixtures, the large complexity of interactions between varieties and between varieties and the environment makes it practically impossible to control all factors in single trials, and the results often include a lot of unexplained variation. As a consequence, experimental results from one study may have low explanatory power, information on underlying interactions may be overlooked, and conclusions may ultimately be insufficient and too limited in scope.

Meta-analysis provides a range of statistical procedures to combine and compare research results in a quantitative manner. Current meta-analysis methodology derives primarily from psychology (Glass 1976) and medicine (e.g. Mann 1990), but has lately been increasingly employed within ecological research (e.g. Gurevitch *et al.* 1992; Osenberg *et al.* 1997; Curtis & Wang 1998; Goldberg *et al.* 1999; Leimu & Koricheva 2006). In meta-analysis, comparable effect measures must be calculated from the selected studies. By combining these, explanatory power is increased and overall measures and relationships might be detected. In this respect, meta-analysis may be a useful tool for summarizing the effects of mixing varieties.

In this study we demonstrate some of the meta-analysis techniques, combining individual trial data from field experiments on variety mixtures to obtain summary estimates of mixing effect, and compare the results with those of a linear model fitted to the full data set.

### Methodology

We used data from 16 field trials of spring barley, including six 3-component variety mixtures and their component varieties (part of the Danish BAR-OF field trials: Østergård *et al.* 2006). The trials were distributed over 4 localities and 4 years and managed according to one of three different growing systems, roughly characterized as: A) conventional experimental field treated with herbicides and insecticides but no fungicides, industrial fertilizer; B) organic experimental field with mechanical harrowing, animal manure; C) organic experimental field, crop undersown with clover grass, no fertilizer or animal manure. In each trial, varieties and mixtures were arranged in  $\alpha$ -design and assessed in 2-3 replicates for grain yield (hkg/ha) and weed ground cover (%; system B only).

For each variety mixture, we estimated the mixing effect with respect to yield and weed cover, respectively, by fitting a mixed linear model to the full set of data consisting of both mixtures and single varieties:

$$Y_{vtrb} = \alpha_v + E_t + F_{tr} + G_{trb} + H_{vtrb},$$

where  $Y_{vtrb}$  is the value recorded for variety  $v$  in miniblock  $b$  within replicate  $r$  of trial  $t$ . The random terms  $E_t$ ,  $F_{tr}$ , and  $G_{trb}$  are assumed independent and normal distributed with zero mean and constant variances. This analysis is referred to as the full model. The mixing effects were then calculated as the contrast in yield or weed cover between a variety mixture and the average of its component varieties.

For the meta-analysis, each trial was considered as a single study, similar to the analyses of Østergård and Willas (2005). An effect of variety mixing was estimated for each mixture in each trial and used as input for the meta-analyses. A version of the described linear model without trial terms was used, and mixing effects were calculated as described above. In each meta-analysis we used a standard fixed effects meta-analysis model (Whitehead 2002), from which a combined meta-estimate of mixing effect ( $\hat{\theta}$ ) was calculated as the weighted mean, weighting trial estimates ( $i = 1, \dots, 16$ ) by the inversed square of their standard error ( $w_i$ ):

$$\hat{\theta} = \frac{\sum \hat{\theta}_i w_i}{\sum w_i}.$$

Standard errors of estimates were calculated as the square root of the inversed sum of weights:

$$se(\hat{\theta}) = \sqrt{\frac{1}{\sum w_i}}.$$

For each combination of mixture and trait, the meta-estimate was tested for equality to 0, using a chi-squared test with one degree of freedom. Further, the overall between-trial heterogeneity in mixing effect on yield and weed suppression was tested using a chi-squared test with 15 and 10 degrees of freedom, respectively (see Whitehead 2000). Also, for yield of each mixture, heterogeneity in meta-estimates among the three management systems was tested, using an ANOVA-analogue of the chi-squared test.

## Results and discussion

The single trial estimates, meta-estimates, and full model estimates of mixing effect are shown in Figs. 1 and 2. In general, the estimates were positive for yield and negative for weed suppression. This implies that the mixtures performed overall better than the average of their component varieties.

According to the meta-analyses, four of the six mixtures produced higher yields than the average of their components, three of them significantly (Fig. 1). Likewise, five mixtures suppressed weeds more efficiently than the average of their components, one of them significantly (Fig. 2).

There were generally only small differences between the estimates of the meta-analysis and the full model (Figs. 1 and 2). However, the full model always gave less accurate estimates than the meta-analysis, as evident from the wider confidence intervals. This was likely due to dubious model assumptions of homogeneous error variance between trials, which will be reconsidered in forthcoming work.

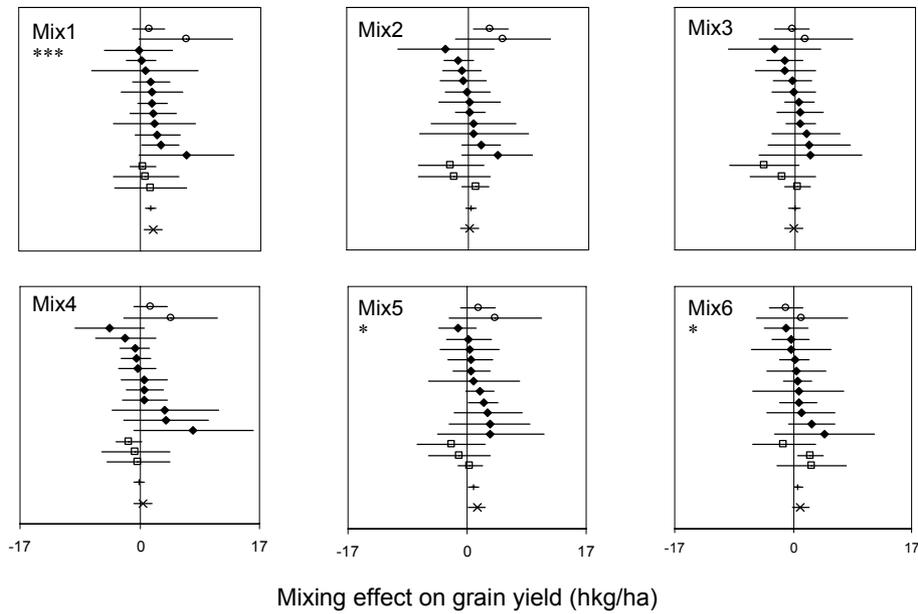
Tests for between-trial heterogeneity of mixing effect were insignificant for all mixtures and both traits (data not shown). However, this test has low statistical power for rejecting homogeneity when there are relatively few studies or, as in the present study, when they are based on small number of replicates. Hence, in forthcoming work, a meta-analysis model will be fitted that takes into account heterogeneity due to random differences between the studies. For yield, there was a general tendency of heterogeneity in meta-estimates among the three management systems (Fig. 1).

## Conclusions

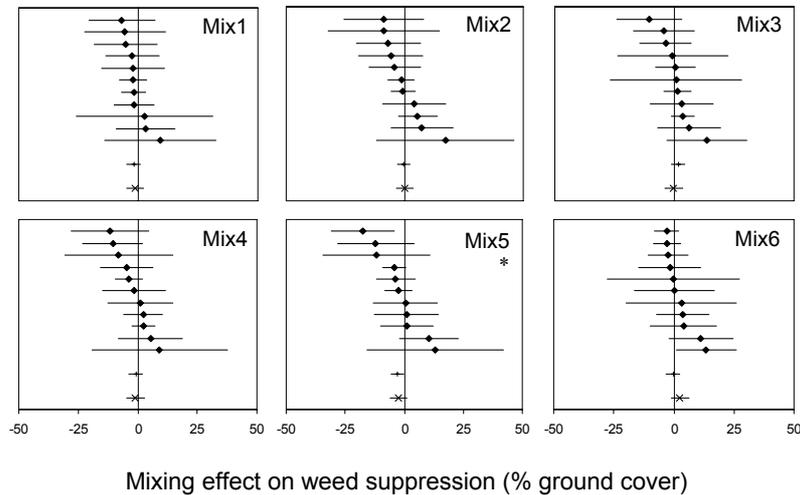
Our results support the general observation that performance of a variety mixture does not simply equal the average of its components. It is also likely that the mixing effect will depend on growing system. Future meta-analyses of variety mixing effects will consider such interactions.

The statistical power of the meta-analyses was sufficiently high to reveal significant effects, despite a relatively small number of trials and considerable variation within and between these trials. The full model, on the other hand, did not fully take into account the variance structure of the data. The analysis of heterogeneity between management systems exemplifies the many useful analytical tools of meta-analysis.

A proper meta-analysis first of all depends on the quality of the data at hand and whether the included studies are representative or biased towards support of specific hypotheses (e.g. Duval & Tweedie 2000). In the effort to produce solid meta-analysis results we have initiated a close collaboration with the researchers in the COST860-SUSVAR network who study variety mixtures.



**Fig. 1.** Estimates of mixing effect on grain yield in variety mixture 1 to 6, including 95% confidence intervals (horizontal lines), for 2 trials of growing system A (circles), 11 trials of growing system B (diamonds), 3 trials of growing system C (squares), meta-estimate (vertical line), and full model (star). Individual trial estimates for each growing system are shown in ascending order. Meta-analysis significance levels:  $P < 0.05$  (\*) and  $P < 0.001$  (\*\*\*)



**Fig. 2.** Estimates of mixing effect on weed cover in variety mixture 1 to 6, including 95% confidence intervals (horizontal lines), for 11 trials of growing system B (diamonds), meta-estimate (vertical line), and full model (star). Individual trial estimates are shown in ascending order. Meta-analysis significance level:  $P < 0.05$  (\*)

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