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A COMPARISON OF THE AMMI METHOD WITH SOME CLASSICAL STATISTICAL METHODS IN PROVENANCE RESEARCH: THE CASE OF THE SOUTH AFRICAN *PINUS RADIATA* TRIALS

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ABSTRACT

Using a five-sites provenance test of *Pinus radiata* in South Africa, three methods (ANOVA, linear regression and graphical representation) to study genotype by site interactions were compared with the Additive Main Effects and Multiplicative Interactions (AMMI) model. For understanding P×E interactions, the AMMI model does not bring new insight over those offered by the three methods mentioned. It does not replace these methods but complements them.

The AMMI method generates more accurate estimators of the treatment means although biased with probably larger sampling variance than the variance of the purely additive model. The main usefulness of the AMMI approach seems to lie in determining the model and the estimator with the best predictive accuracy, thus ensuring greater genetic gain if that estimation was used for selecting the best provenances.

Key words: *Pinus radiata,* P×E interaction, AMMI method, comparison with ANOVA, linear model

INTRODUCTION

Recent years have seen the publication of a number of papers presenting some applications of the so-called Additive Main Effects and Multiplicative Interaction (AMMI) model to a number of multi-sites plant breeding experiments (*e.g.*, GAUCH 1988, GAUCH & ZOBEL 1988, CROSSA *et al.* 1990, CROSSA *et al.* 1991). The papers claim to a varying degree that the AMMI model is effective for: (i) understanding the genotype × environment interaction, (ii) improving the accuracy of yield estimates, (iii) increasing the probability of selecting the genotypes with highest yields, and (iv) increasing the flexibility and efficiency of experimental design.

This paper examines whether the application of the AMMI method model can bring the same benefits as those claimed for plant breeding experiments when that technique is applied to provenance trials.

Multi-sites forest tree breeding experiments are rare: the series of *Picea abies* provenance trials established in Southern Sweden (WELLENDORF *et al.* 1986), of *Pinus radiata* progeny trials in Australia (MATTHESON & RAYMOND (1984) and in South Africa of the six *Pinus radiata* provenance trials (FALKENHAGEN 1991) seems to be the only multi-sites experiments reported on. Because of my intimate knowledge of the South African *Pinus radiata* trials, the results of these trials will be used to compare the following three methods: (i) across sites analysis of variance, (ii) linear regression method, and (iii) simultaneous plotting of the provenance means for all other trials against the ranking of the provenances at one trial for each trait (graphical representation of the interaction between trials), with the AMMI technique.

MATERIAL AND METHODS

The genetic variation in height, diameter at breast height, stem form, crown form, and resistance to the aphid *Pineus pini* at eight years between 10 provenances of *Pinus radiata* in five trials planted in South Africa was used to compare the AMMI technique with the following three approaches:

- across-sites analyses of variance using a completely random model to test the effects, complemented with plotting of the provenances over the characteristics of the place of origin for each trial,
- regression of mean of each provenance on the trial average (the so-called linear regression or Finlay-Wilkinson method). Provenances with average stability should have a regression coefficient of one. The regression coefficient of each provenance, once significantly different from zero, was also tested for equality with one. The dispersion of the provenance means around the regression lines was examined at visually,

Table 1 Names and geographical coordinates of *Pinus radiata* provenances studied (according to FALKENHAGEN 1991)

Australia Stock Numbers	South Africa Stock Numbers	Population	Subpopulation	Latitude	Longitude	Altitude (m)	Number of trees collected
S12586	30464	Ano Nuevo	Coastal strip	37.10	122.28	20	70
S12585	30465	Ano Nuevo	Inland Central	37.10	122.25	200	40
S12588	30467	Ano Nuevo	Inland Northern	37.13	122.30	140	20
S12590	30468	Monterey	Coastal sand dunes	36.62	121.95	30	55
S12589	30469	Monterey	Monterey-Delmonte	36.58	121.87	45	37
S12592	30470	Monterey	Huckleberry Hill	36.58	121.92	135	35
S12591	30471	Monterey	Jacks Peak Park	36.55	121.87	200	61
S12596	30474	Cambria	Pico Creek	36.62	121.15	75	26
S12597	30475	Cambria	Cambria Town	35.57	121.10	75	49
S12557	30477	Canberra	Guadalupe hybrid				

- visual representation of provenance by site interaction by plotting each provenance against the rank of provenances at one trial, for each trait and each other trial.

A detailed description of the trials, the origin of the provenances, the traits measured as well as the analysis of variance and interpretation of these trials are to be found in the paper by FALKENHAGEN (1991). In Table 1 the names and their corresponding geographical coordinates are given. The five trials studied were Bergplaas, Hogsback, Kluitjeskraal I, Kruisfontein and Witfontein, the sixth trial mentioned in FALKENHAGEN (1991) was destroyed by fire. In order to apply the AMMI techniques ten provenances only were used, those common to the five trials and with a complete set of replications. In other words, no missing plot was allowed. Because most trials contained 1 to 10 replications a completely random design was adopted, instead of a randomized complete block design. In spite of this restriction, the material used is nearly identical to that used in Falkenhagen's paper. For instance in the ANOVAs 262 degrees of freedom were used while the AMMI model used 249.

The AMMI model was

$$Y_{ijr} = \mu + \alpha_i + \beta_j + \sum_{k=1}^{n} \lambda_k \varphi_{ik} \delta_{jk} + \rho_{ij} + \varepsilon_{ijr} \qquad [1]$$

where: μ is the grand mean, α_i is the provenance mean deviation (or provenance effect) i with $i = 1, \ldots\ldots, g$, β_j is the site effect j with $j = 1, \ldots\ldots, e$, λ_k is the singular value for axis k with $k = 1, \ldots, n$, φ_{ik} is the provenance eigenvector value for the axis k, δ_{jk} is the site eigenvector for axis k, ρ_{ij} is the residual, ϵ_{ijr} is the error with $r = 1, \ldots, 5$ replications, $\epsilon_{ijr} = Y_{ijr}$ minus the mean μ_{ij}, ϵ_{ijr} is distributed normally with mean zero and standard deviation σ.

The least squares fit for balanced data is obtained by first fitting the additive part of the AMMI model (μ, α_i and β_j)with the ordinary analysis of variance and then applying the singular value decomposition of the matrix of residual $(y - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j)$ in order to obtain an estimation of the parameters of the multiplicative part (JOHNSON & GRAYBILL 1972, and GAUCH 1990).

Postdictive assessment was done by approximate F-test by comparing each axis component mean square with the error mean square, using the method of GOLLOB (1968).

Predictive assessment was carried out according to GAUCH and ZOBEL (1988). The data were split into two subgroups: modeling data and validating data. For each provenance – site combination, four or three replicates were chosen at random for modeling and the other or the two other ones were respectively reserved for validation. In both cases fifty calculations were done and the average square root of the mean squared difference between predicted and validation data calculated (RMSPD).

The following models were fitted in all cases:

- AMMI 0 – additive main effects without interaction;
- AMMI 1 – additive main effects plus provenance by site (PS) interaction effect for axis 1;
- AMMI 2 – additive main effects plus interaction effect for axes 1, and 2;
- AMMI 3 – additive main effects plus interaction effect for axes 1, 2, and 3;
- AMMI 4 – additive main effects plus interaction effect for axes 1, 2, 3, and 4;
- CELL MEANS – mean of the replications selected at random for modelling.

The approximate number of replications needed for the CELL MEANS model to equal the performance of the best AMMI model was also calculated. This number enables to estimate how efficient the best AMMI model is in "saving replications".

Residuals for the AMMI 1 model were calculated and examined. The biplots corresponding to that model were also constructed and interpreted, although in most cases AMMI 1 was not the best predictive model but AMMI 0. To judge the validity of the AMMI method it was considered as crucial that the biplots be interpreted according to known genecological principles as exposed by, for instance, STERN & ROCHE (1974). Although the classical analysis of variance did not detect large provenance by site interaction, it was deemed useful to apply the AMMI method in order to see whether that method shows any useful result or any artifact. Thus whether the interaction was significant or not a biplot was constructed and analyzed as advised by GAUCH (1990). All the AMMI calculations were realized by using the MATMODEL, version 2.0 programme (GAUCH 1990).

RESULTS

Results of the classical approaches

There was a small significant site by provenance interaction for height and aphid resistance. The component of variance for the interaction was 4.2% and 5.5% of the total variance, respectively. However, for height the provenance effect contributed only 1.1% while for aphid resistance it contributed 7.5% of the total variance. Thus, compared to the most important effect (provenance) the interaction was not negligible in relative terms. For height it made the selection of provenance overall sites nearly impossible as the interaction component in absolute value was 3.73 times the provenance component variance. Plotting of the mean aphid resistance of the provenances over the rank of the corresponding provenances at Kluitjeskraal I shows that the Hogsback site was responsible for that interaction with the Ano Nueavo population showing better resistance. Aphid infestation was its worst at Hogsback (average 4.35) as opoosed to Kluitjeskraal I (4.99) (FALKENHAGEN 1991).

The results of regreesing each provenance mean on the trial mean for all the traits studied at eight years, are as follows. Height and diameter did not show any regression line which had a regression coefficient significantly different from one, partly because of the small number of degrees of freedom available. For average volume per tree, most provenances showed average stability. However, one provenance (30475) from Cambria had a regression coefficient highly significant different from one.

For aphid resistance the regression analyses confirmed the across sites analyses of variance and indicated clearly what provenances were unstable. In practical terms, most provenances and controls appeared to have average stability as expressed by their linear regression for the growth traits studied however (FALKENHAGEN 1991)

Results of the AMMI method

The residuals – not to be confused with the error – were deemed normally distributed in an acceptable way with the discrete variables (stem form, etc.) slightly less normally distributed. The error is unlikely not to be distributed normally. These facts will thus not be mentioned in what follows.

Height

Table 2 shows that the provenance and site effects and the interaction axis 1 are highly significant.

Table 3 shows that the additive model without interaction has the smallest RMSPD. The number of effective replications is 7.66 and 6.31 when one or two replications were predicted respectively. Thus an increase in precision equivalent to that from adding one replication was obtained by using the additive model for

Table 2 Summary of the analysis of variance based on the AMMI model for the traits studied at eight years

Source of variance	D.f.	Height (m)		Diameter (cm)		Stem form (classes)		Crown form (classes)		Aphid resistance (classes)	
		M.S.	P	M.S.	P	M.S.	P	M.S.	P	M.S.	P
Treatment	49	15.0094	0.0000	33.8756	0.0000	0.4530	0.0000	3.2731	0.0000	0.3790	0.0000
Provenance (P)	9	2.3351	0.0061	6.0307	0.0212	0.1907	0.0209	0.2586	0.0012	0.3421	0.0000
Site (S)	4	167.4731	0.0000	390.6327	0.0000	4.6723	0.0000	38.2522	0.0000	3.0734	0.0000
P×S	36	1.2376	0.0732	1.1972	0.9975	0.0497	0.9716	0.1401	0.0102	0.0888	0.0036
Axis 1	12	2.1240	0.0059	2.5866	0.4894	0.0520	0.8318	0.3124	0.0000	0.1881	0.0000
Residual	24	0.7945	0.5949	0.5025	0.9999	0.0486	0.9471	0.0539	0.8820	0.0392	0.7000
Error	200	0.8775		2.6975		0.0852		0.0812		0.0474	
Total	249										

election.

A biplot for axis 1 and 2 were constructed and interpreted. The first biplot (Figure 1) shows all sites except Bergplaas contributing large interaction scores while the provenances tend to be grouped according to population but the populations of Cambria and Monterey overlapped and were not ranked in geographical order. In other words, no genecological interpretation or any other biological interpretation was possible. The second biplot was quite different from the first one and led to even less interpretation with the three populations overlapping. In this case no insight was given by AMMI while the other classical methods led to some genecological interpretation. Figure 2 shows clearly the linear or curvilinear relationship of height with latitude of place of origin in other words with well-known macroclimatic changes.

Diameter

Table 2 shows that the provenance and site effects are significant but that there is no significant interaction effect.

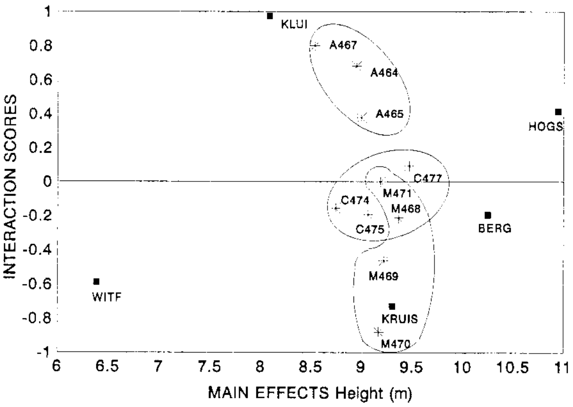

Figure 1 Biplot for the unadjusted means for height and the first eigenvector of 10 provenances of *Pinus radiata* (*) and five sites (■). The numbers of the provenances are as in Table 1. The names of the sites have been abbreviated as follows: BERG for Bergplaas, HOGS for Hogsback, KLUI for Kluitjeskraal, KRUIS for Kruisfontein, WIT for Witfontein

Table 3 shows again that the additive model without interaction has the smallest root mean square prediction differences. The number of effective replications was

Table 3 Root mean square prediction differences (RMSPD) for the traits studied at the age of 8 when one or two replications are predicted

Model	Height		Diameter		Stem form		Crown form		Aphid resistance	
	Number of replications predicted									
	1	2	1	2	1	2	1	2	1	2
AMMI 0	0.99604	1.0082	1.6450	1.6622	0.2928	0.2955	0.3071	0.3136	0.2344	0.2452
AMMI 1	1.00981	1.0436	1.7217	1.7990	0.3129	0.3183	0.3064	0.3152	0.2374	0.2517
AMMI 2	1.01801	1.0658	1.7959	1.8691	0.3212	0.3289	0.3176	0.3265	0.2359	0.2497
AMMI 3	1.03893	1.0826	1.8226	1.9003	0.3229	0.3338	0.3193	0.3304	0.2371	0.2512
AMMI 4	1.04247	1.0878	1.8401	1.9124	0.3222	0.3354	0.3202	0.3311	0.2383	0.2522
CELL MEANS	1.04247	1.0878	1.8401	1.9124	0.3222	0.3354	0.3202	0.3311	0.2383	0.2522

Table 4 Comparison of the ability of different techniques used in detecting interaction between sites and provenances at the age at 8 years

Characteristics studied	ANOVA models	Linear regression	Graphical representation	AMMI method	Best AMMI model selected
Height	Yes	No	Yes	Yes	AMMI 0
Diameter	No	No	Yes	No	AMMI 0
Stem form	No	No	No	No	AMMI 0
Crown form	No	No	Yes	Yes	AMMI 1
Aphid resistance	Yes	Yes	Yes	Yes	AMMI 0

Explanation: Yes – interaction significant, No – interaction not significant

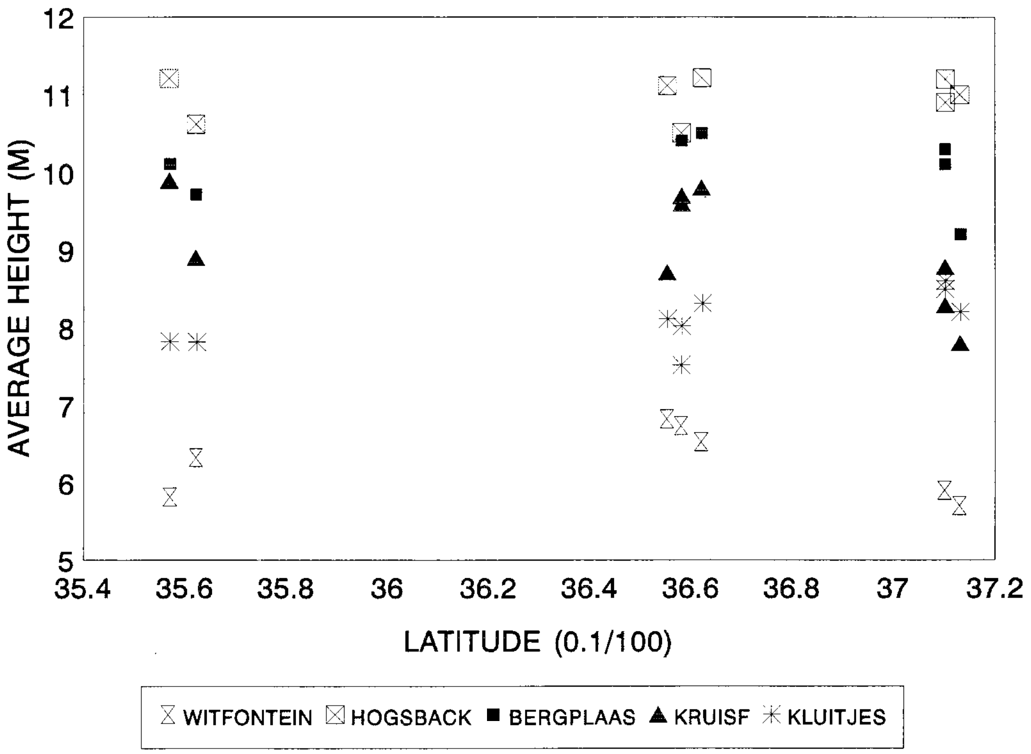

Figure 2 Plotting of the provenance average height on latitude of place of origin for the five sites studied. The pattern of variation varies markedly from trial to trial as well as the average differences between the three populations.

316.61 and 41.13 respectively, when one or two replications were predicted.

Stem form

Table 2 shows that the provenance and site effects were significant but not the provenance by site interaction, or any axis. Nevertheless a biplot with the first axis was constructed and analyzed.

Table 3 shows that the additive model without interaction again has the smallest root mean square prediction differences. The number of effective replications was 141.71 and 39.26, respectively, when one or two replications were predicted.

The provenances were grouped into clusters with the three populations barely distinct. The Canberra provenance had the largest interaction score (–0.37). No interpretation was possible for the sites. Note that the populations were shown different by the ANOVAs according to the trial.

Crown form

Table 2 shows that the provenance and site effects, the interaction effect and the first axis were highly significant.

Table 3 shows that the AMMI 1 model had the smallest root mean square prediction difference when one replication was predicted and that the additive model was the best when two replications were predicted. The number of effective replications was 6.41 and 4.75, respectively.

Although there is a contradiction between the results of the two validating procedures, the two first biplots were constructed and interpreted.

The first biplot indicates that the three populations were fairly distinct in interaction scores from Ano Nuevo provenance 30467 with a score of +0.46 to a Cambria provenance with a score of –0.47 (provenance 30474). The populations were ranked in the geographical order. Thus some genecological interpretation was possible. The Hogsback site was quite distinct from the other sites by its large interaction score (+0.82), the other having small and negative interaction scores.

The second biplot was very different from the first one with the three populations largely mixed-up and the site scores uninterpretable. The second biplot seemed to be largely "noise".

Aphid resistance

Table 2 shows that the provenance and site effects were highly significant together with the interaction and its first eigenvector. Two biplots were nevertheless constructed and analyzed with the first two axes.

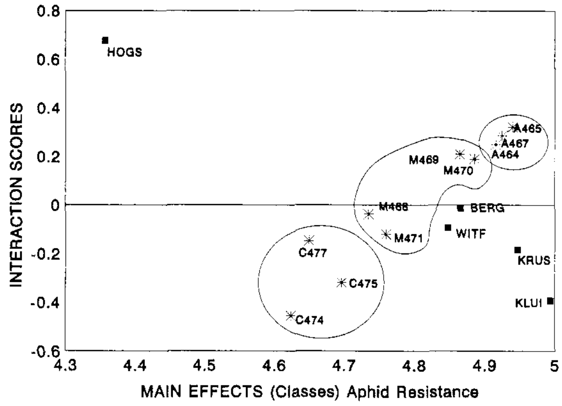

Figure 3 Biplot of the unadjusted means for aphid resistance and the first eigenvector of 10 provenances of *Pinus radiata* (*) and five sites (▬). The abbreviations are as in Fig. 1.

Table 3 shows that the additive model has the smallest root mean square prediction differences with the number of effective replications of 6.29 and 3.73, respectively, when one or two replications were predicted.

The biplot for the first axis (Figure 3) shows clear separation of the three populations with their rank corresponding to their geographical position. The Cambria population had the largest negative score and the Ano Nuevo population the largest positive (–0.48 and +0.28, respectively). The sites were aligned along an axis negative – positive scores, with Hogsback having the largest positive score (+0.68) and Kluitjeskraal the largest negative one (–0.40). The other sites had small interaction scores. The classical methods used here indicated that Hogsback presented the most abnormal image as far as ranking of the provenances for aphid resistance was concerned. Thus a classical genecological interpretation of the first biplot was possible. The second biplot showed mainly small, probably, negligible interaction scores, with the populations barely distinct and distorted and the sites showing uninterpretable scores.

DISCUSSION AND CONCLUSION

When there was a significant interaction between site and provenance, the AMMI method showed a pattern of interaction scores which enabled some genecological interpretation in the sense that the three fundamental populations appeared as distinct clusters often ordered as in the geographical space. However, that grouping was detected easily by the classical methods used in provenance research like ANOVA models, plotting against geographical coordinates or graphical representation of the provenances ranked over the different sites. Similar comments are possible for the results of the application of AMMI to trials of soybean (ZOBEL *et al.* 1988), maize (CROSSA *et al.* 1990), and wheat (CROSSA *et al.* 1991).

Similar comments apply to the ordination of the sites according to their interaction score. Furthermore there was often a contradiction between the largest interaction score of the provenance for height at eight years for instance and the stability shown by the same provenances when linear regression techniques have been used.

The advantage of the Finlay-Wilkinson approach is that it gives a visual representation of the stability of the genotypes over the sites as well as some simple statistics to quantify that stability. The AMMI technique was applied to South African maize trials and gave the same conclusions (FALKENHAGEN *et al.* prepared). There is a

Table 5 Sampling variance of the different estimators of the treatment effects calculated by the different AMMI

Estimator	Sampling variance
Cell Means $\bar{X}_{ij}=\sum_{k=1}^{r}\frac{X_{ijk}}{r}$	$\frac{\sigma^2}{r}$
Additive Model (AMMI 0) $\hat{X}_{ij.}=\bar{x}_{i..}+\bar{x}_{.j.}+\bar{x}_{...}$	$\frac{\sigma^2}{ger}\times(g+e-1)$
AMMI Model $\hat{X}_{ij.}=\bar{x}_{i..}+\bar{x}_{.j.}+\bar{x}_{...}+\lambda_i\varphi_{ij}\delta_1$	variance larger than $\frac{\sigma^2}{ger}\times(g+e-1)$

g – number of provenance studied
e – number of sites tested
r – number of replications used

tendency for the maize cultivar with the largest score in absolute value to be judged unstable by the Finlay-Wilkinson method but the relationship is weak and does not indicate whether the cultivar under-reacts or over-reacts over the range of site productivity studied (see FALKENHAGEN *et al.*op. cit.). The size of an interaction scores does not indicate necessarily the nature and importance of the interaction caused by the element concerned. Other techniques must still be used and genetical or other consideration still used.

There is not test of significance for these scores and I suspect that in some cases, an interaction score of 0.5 (in absolute value) might not be significantly different from zero, because I could not detect any interaction for the elements concerned using other methods.

In most cases, when purely additive model (the AMMI 0) was selected as the best predictive model, no interaction was detected using ANOVA techniques, the only exception being height and aphid resistance at eight years (Table 4).

Although the most predictively accurate model was mainly the additive model without interaction on the basis of the root mean square prediction differences there was often very little difference between RMSPD values calculated. Thus the practical significance of choosing the best predictively accurate model remains to be investigated.

Another problem which should be solved is the estimation of sampling variance of the different estimators generated by different AMMI models. If the model with the best predictive accuracy is the AMMI 0, (the purely additive one), then it has a smaller sampling variance than the Cell Means model (Table 5). Thus the application of some ranking and selection method should be more precise and more useful (see, for instance, GIBBONS *et al.* 1977). However, what happens when a more complicated model (AMMI 1 or AMMI 2) is used to estimate the treatment means? CHADOEUF and DENIS (1991) have calculated the variance – covariance of the parameters constituting the multiplicative term but not the variance of the estimator. I have tried some Monte Carlo simulation and it looks as if the sampling variance of AMMI 1 is larger than that for the purely additive estimator, although most probably it will be smaller than the sampling variance of the Cell Means (personal communication of Dr. D. Bradu, University of South Africa).

All AMMI estimators except the Cell Means are biased because of the so-called Stein effect but more accurate in predicting the cell treatment than the Cell Means (GAUCH 1990b), thus if they have a smaller sampling variance, their use should be more precise and more accurate.

Although the AMMI technique can be used as a exploratory technique it does not replace the other statistical methods used in provenance research which present other ways to look at the same set of data.

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