Genotype $\times$ Environment Interaction for Resistance to Spider Mites in *Fragaria*

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ABSTRACT. Genotype $\times$ environment interaction for resistance to the twospotted spider mite (*Tetranychus urticae* Koch) of eleven clones of *Fragaria* L. sp. (strawberries) grown in six environments throughout the United States was examined using two multivariate analysis techniques, principal coordinate analysis (PCA) and additive main effect and multiplicative interaction (AMMI). Both techniques provided useful and interesting ways of investigating genotype $\times$ environment interaction. PCA analysis indicated that clones X-11 and E-15 were stable across both low and high environments for the number of spider mites per leaflet. The initial AMMI analysis showed that the main effects of genotype, environment, and their first-order interaction were highly significant, with genotype $\times$ environment interaction due mainly to cultivar 'Totem' and environment FL94. A second AMMI analysis, which excluded 'Totem' and FL94, showed that the main effects of the remaining genotypes, environments, and genotype $\times$ environment interaction were also highly significant. AMMI biplot analysis revealed that FL93 and GH93 were unstable environments, but with opposite interaction patterns; and GCL-8 and WSU2198 were unstable genotypes with similar interactions that were opposite those of WSU 2202.

The twospotted spider mite, *Tetranychus urticae* Koch, is a major pest of strawberry (*Fragaria* L. sp.) in the United States and Europe (Galletta and Bringham, 1990; Rosati, 1991). Identification of *Fragaria* clones resistant to spider mites, as well as breeding for spider mite resistance in strawberry, was reviewed by Hancock et al. (1991). Hancock et al. (1996) reported resistance of strawberry clones to spider mites to be moderately to highly heritable, but Shanks et al. (1995) detected significant genotype $\times$ environment effects in a study that examined resistance of eleven *Fragaria* clones grown in three widely separated locations. Genotype $\times$ environment interactions can have important implications for breeding plans (Wright, 1976), and therefore the initial objective of this study was to expand on the analysis of Shanks et al. (1995).

Response of a clone in E different environments may be conceptualized as a pattern in E dimensional space, with the coordinate of an individual spatial axis being the number of spider mites per leaflet of the clone in one environment. Since clone responses are multivariate rather than univariate (Lin et al., 1986;

Van Oosterom et al., 1993), multivariate techniques are preferable, as they are usually more effective than linear regression models for explaining genotype $\times$ environment interactions (Gauch and Zobel, 1988; Nachit et al., 1992; Zobel et al., 1988).

The additive main effect and multiplicative interaction (AMMI) model with prediction assessment was proposed for analysis of two-way tables. In this model, main effects are first accounted for by an analysis of variance; thereafter the interaction is analyzed by a principal component analysis (Gauch, 1988; Gauch and Zobel, 1988). The optimum number of interaction principal component axes (IPCA) to be retained in the model, to obtain the most accurate estimation for number of spider mites per leaflet, can be determined by two different assessments referred to in the literature as predictive and postdictive. The predictive assessment splits the data set into a part for model validation (Gauch, 1988; Gauch and Zobel, 1988; Krzanowski, 1983; Wold, 1978). Postdictive refers to a different method of validation which uses an F test to identify the significance of each IPCA.

Principal coordinate analysis (PCA) is a multidimensional scaling or ordination technique used to represent the structure or pattern that may be present in the observed data matrix and provides a geometrical configuration of points in a low-dimensional space (Gordon, 1980). The distance between points in the low-dimensional diagram will reflect the relationship between items in the original observed matrix. Similar items are repre-

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sented by points that are close together, while dissimilar items are represented by points distant from each other. Therefore, our specific objectives in this study were to 1) determine the magnitude of the genotype $\times$ environment interaction effects, 2) identify clones that performed well and remained stable under different environmental conditions, and 3) examine and compare the results obtained with AMMI and PCA.

Materials and Methods

Plant material and environments

Ten *Fragaria* clones (mostly *F. chiloensis* Duch. and hybrids of *F. chiloensis* and *F. xananassa* Duch.) and 'Totem', a cultivar known for susceptibility to spider mites (Shanks and Barritt, 1975, 1980), were grown in six environments: 1) a field at Dover, Fla., in 1993 (FL93); 2) a field at Dover in 1994 (FL94); 3) a greenhouse at Vancouver, Wash., in 1993 (GH93); 4) a greenhouse at Vancouver in 1994 (GH94); 5) a field in Vancouver in 1993 (WA93); and 6) a field at Watsonville, Calif., in 1993 (CA93). Clones were replicated four times in a randomized complete-block design. Mite populations developed naturally in the field and greenhouse. Field trials were evaluated by removing 10 trifoliate leaves from each plot every 2 weeks, counting the mites on the underside of the leaves, and pooling the numbers for each plot. Greenhouse trials were evaluated by removing one leaflet from each of three trifoliate leaves on each plant every 2 weeks, counting the mites, and pooling the numbers for each plant. This paper is based on further analysis of the seasonal mean data collected by Shanks et al. (1995).

Statistical methods

AMMI ANALYSIS. The AMMI model is $Y_{ij} = \mu + g_i + e_j + \sum_k \lambda_k \gamma_{ik} \sigma_{jk} + \varepsilon_{ij}$, where Y_{ij} is the number of spider mites per leaflet of the i^{th} genotype in the j^{th} environment; μ is the grand mean; g_i and e_j are the genotype and environment deviations from the grand mean, respectively; λ_k is the eigenvalue of the PCA axis k ; γ_{ik} and σ_{jk} are the genotype and environment principal component scores for axis k ; N is the number of principal components retained in the model; and ε_{ij} is the error term. Environment and genotype PCA scores are expressed as unit vector times the square root of λ_k (i.e., environment PCA score = $\lambda_k^{0.5} \sigma_{jk}$; genotype PCA score = $\lambda_k^{0.5} \gamma_{ik}$) (Zobel et al., 1988).

Gauch and Zobel (1988) created the term postdictive (symmetrical to predictive) to measure the level of success of the prediction. Postdictive success was measured by comparing each principal component's mean square with the pooled within-environment error mean square. Those PCA axes that were not significant were pooled into a residual term.

Predictive assessment was carried out by the cross-validation procedure described by Gauch (1988). The data were split into two subgroups: model data and validation data. For each treatment (i.e., genotype and environment combination), two replicates were selected at random to be modelled by AMMI, and the other two were reserved as validation observations. The sum of the squared differences between the model's fitted values and validation data over genotypes and environments is divided by the number of validation observations, and its square root is taken to give the root mean square predictive difference (RMSPD). Smaller values of RMSPD indicate good predictive success.

Five models were fitted to the data. The first is the AMMI0 model which estimated the additive main effects (i.e., genotypes and environments) without considering interaction; the second, AMMI1, combined the main effects from AMMI0 with interaction effects

estimated from the first interaction principal component axis (IPCA1). The third and fourth model, AMMI2 and AMMI3, considered main effects plus two and three interaction principal component axes, respectively. The fifth model, AMMI4, with four PCA axes, is the full model, and completely specifies the data matrix and equals the average of the two replicates selected at random for modelling.

PCA. PCA was proposed by Westcott (1987) and used by Crossa (1988). As detailed by Crossa (1988), the similarity measured between any given pair of genotypes [$S_i(X, Y)$] indicates the proximity of its average to H_i and the dissimilarity [$D_i = 1 - S_i(X, Y)$] indicates the proximity of its average to L_i .

$$S_i(X, Y) = [H_i - (X_i + Y_i)/2] / (H_i - L_i)$$

where H_i = mean number of spider mites per leaflet for the most heavily infested genotype in environment i ; L_i = mean number of spider mites per leaflet for the least heavily infested genotype in environment i ; X_i = mean number spider mites per leaflet of genotype X in environment i ; and Y_i = mean number of spider mites per leaflet of genotype Y in environment i .

When more than one environment is considered, the dissimilarity between genotypes X and Y is defined as the average of $D_i(X, Y)$ across environments. Smaller values for D indicate greater proximity to H , and higher values for D indicate greater proximity to L . Crossa (1988) stated that the analysis determines a point from which all clones radiate. This point, with minimum value for D , is the center of the scattergram (see Westcott, 1987, on minimum spanning tree). Therefore, clones with small values for D are represented by points clustered near the center of the scattergram and clones with high values for D are represented by points far from the center. The environments are first ranked in descending order of mean number of spider mites per leaflet and the low and high environments are then analyzed in cycles. Clone performance is first analyzed for the lowest environment (cycle L1); the second cycle (L2) involves analyzing the two lowest environments, and so on (Table 1). The same procedure is followed for the highest environments (cycle H1, H2, ...etc.). Clones with low numbers of spider mites per leaflet are represented by points located away from clones that have high numbers of spider mites per leaflet. The stable clones are the ones that are consistent over cycles (Westcott, 1987).

Results

AMMI ANALYSIS. The first AMMI analysis, with all clones and environments, showed that all sources of variation, including the main effects of genotype and environment and their first-order interaction, were highly significant ($P < 0.01$) (data not shown).

Figure 1 is a graphical representation or biplot of genotype $\times$ environment interaction, which shows main effect means on the abscissa and IPCA1 values of both clones and environments, simultaneously, on the ordinate (Kempton, 1984). Displacement

Table 1. Environments included in each cycle of the principal coordinate analysis performed on the number of spider mites per leaflet for each of 11 *Fragaria* clones.

Cycle	Environment		Total environments included
	Environment included	included mean	
L1	WAF93	8.906	WAF93
L2	CA93	16.053	WAF93 + CA93
L3	GH94	19.810	WAF93 + CA93 + GH94
L4	GH93	44.205	WAF93 + CA93 + GH94 + GH93
H1	FL93	47.149	FL93
H2	FL94	138.734	FL93 + FL94

Fig. 1. Biplot of principal component analysis (IPCA1) axis 1 against the number of spider mites per leaflet for 11 clones grown at six environments. The vertical line represents the grand mean of the experiment.

along the abscissa reflects differences in main effects, whereas displacement along the ordinate illustrates differences in interaction effects. Genotypes with IPCA1 values close to zero show wider adaptation to the tested environments. Genotype and location combinations with IPCA1 scores of the same sign have positive specific interactions, whereas combinations with opposite signs have negative specific interactions.

Figure 1 shows that genotype $\times$ environment interaction was due mainly to cultivar 'Totem' and environment FL94. They had IPCA1 scores that were highly negative, combined with the highest mean number of spider mites per leaflet. The rest of the clones and environments are clustered on the left side of the graph. In order to investigate the interactions within this cluster, 'Totem' and FL94 were removed from the data set and a second AMMI analysis was performed.

This second AMMI analysis showed that environments, clones, and genotype $\times$ environment interaction were also highly significant ($P < 0.001$) and accounted for 34.6%, 10.1%, and 19.6% of the total sum of squares (SS), respectively (Table 2). The criterion of postdictive success for AMMI recommended including the first interaction PCA axis in the model because only IPCA1 was significant. The model accounted for 94.6% of the treatments SS (environment + clone + genotype $\times$ environment) (Table 2).

Predictive assessment AMMI1, the model including only the first IPCA, had the lowest value for RMSPD and was hence most predictive (Table 3). Results of the second AMMI analysis are represented graphically in Fig. 2. Three groupings of clones are evident from Fig. 2. Group 1 includes clones X-11 and CL-5, that is, the most spider mite resistant clones. They show a similar mean number of spider mites per leaflet and a homogeneous IPCA1 close to zero.

Group 2 consists of clones A-16, E-15, G-9, RCP-37, M-1, and WSU-2202. Their IPCA1 scores are positive (M-1's value is low, while that of WSU-2202 is relatively high) and they show a similar

mean near the grand mean. Group 3 consists of clones GCL-8 and WSU-2198. These clones show the highest mean number of spider mites per leaflet and their interaction scores are the most negative.

The environments show much variability in both main effects and interactions. Two environments, FL93 and GH93, were unstable, but with opposite interaction patterns; whereas the other three environments, CA93, WAF93, and GH94, had relatively small interactions (Fig. 2).

PRINCIPAL COORDINATES ANALYSIS. Rather than including all six scattergrams, the stability patterns of the clones are described in the text and only two scattergrams (Figs. 3 and 4), corresponding to cycles L4 and H1, are presented.

Table 4 shows the mean length of the minimum spanning tree, including only the lowest environments (MEDLI), the highest environments (MEDHI), and all environments (MEDTO) (Cubero and Flores, 1994).

PCA of the four environments (WAF93, CA93, GH94, and GH93) with a low number of spider mites per leaflet showed a clear

pattern of stability for clones CL-5, X-11, and WSU 2202 (Table 4: MEDLI, values in bold type). Of these, clone WSU 2202 was closer to the center in L1 cycle, while clones CL-5 and X-11 appeared very stable in all the L cycles.

Table 2. Additive main effects and multiplicative interaction (AMMI) analysis of variance for the number of spider mites per leaflet, including the first two interaction principal component analysis (IPCA) axes (AMMI analysis without 'Totem' and FL94).

Source of variation	df	MS ^z	R ^{2y}
Treatment	49	935	64.3
Environment (E)	4	6169.11***	34.6
Clone (C)	9	798.19***	10.1
C $\times$ E	36	387.83***	19.6
IPCA1	12	940.88***	82.4
IPCA2	10	154.38 ^{NS}	11.8
Residual	14	61.26 ^{NS}	5.8
Replications within E	15	493.19***	10.4
Error	135	133.45	25.3
Total	199	357.97	

^zMean square. Hypothesis based on a fixed model. The significance of the AMMI models is based on postdiction.

^yFraction of sum of squares associated to each term or interaction.

^{NS,***}Nonsignificant or significant at $P = 0.05, 0.01$, or 0.001 , respectively.

Table 3. Average root mean square predictive difference (RMSPD) (number of spider mites per leaflet) for 5 additive main effects and multiplicative interaction (AMMI) models based on 25 randomizations.

Model	RMSPD
AMMI0	17.16
AMMI1	14.33
AMMI2	14.66
AMMI3	14.72
AMMI4 ^z	14.68

^zFull model.

Fig. 2. Biplot of principal component analysis (IPCA1) axis 1 against the number of spider mites per leaflet for ten clones (without 'Totem') grown at five environments (without FL94). The vertical line represents the grand mean of the experiment.

Clones X-11, GCL-8, E-15, and WSU 2198 were the remotest points (and therefore the most stable) in the two H (environments

Fig. 3. Plot of the first two principal axes from a principal coordinate analysis of a set of *Fragaria* clones in six environments, in cycle L4. Part of the minimum spanning tree is superimposed on the plot, the distances between clones (e.g., 'Totem' - X-11 = 0.49) being the dissimilarities.

FL93 and FL94) cycles (Table 4; their MEDHI values are in bold). Of these clones, X-11 and GCL-8 were stable in both H cycles, while X-11 and E-15 showed consistent patterns of stability in L and H cycles (Table 4; their MEDTO values are in bold).

Discussion

Results obtained with the second AMMI analysis indicate that clones X-11, CL-5, and M-1 were the most stable because their IPCA1 scores are near zero. Clone M-1, however, shows a mean response near the grand mean, while X-11 and CL-5 showed the smallest mean response, proving to be the most widely adapted for low number of spider mites per leaflet (Fig. 2).

PCA (or the spatial method) indicates that clones X-11 and E-15 were stable across both low and high environments for the number of spider mites per leaflet and were by far the most spider mite resistant clones in the study. Four other

clones exhibited a moderate degree of stability: CL-5, A-16, M-1, and WSU 2202 (Table 4). These results agree largely with those obtained with the second AMMI analysis.

Results obtained with the spatial method are useful for comparing the merits of different clones, and indicate which ones are capable of stability across environments.

Our study revealed that PCA might be more straightforward than AMMI analysis when there are values (such as those of 'Totem' and FL 94) that are conspicuously separated from the majority of other values. Crossa (1988) and Hill and Bagler (1983) concluded that regression analysis should be used with caution when one of the environments is atypical. A weakness of AMMI is that when there are atypical values, this analysis can show a distorted view of both clones and environments in the biplot. A strength of AMMI, however, is that it distinguishes different kinds of instability by different directions in the biplot. Consequently, AMMI can show which genotypes perform best in which environments, and can group the environments into mega-environments in which a given genotype wins (or nearly wins). More specifically, AMMI says that FL93 and GH93 were both unstable environments, but they had opposite interaction patterns; and, correspondingly, GCL-8 and WSU-2198 were unstable genotypes with similar interactions that are opposite of WSU-2202.

It should be noted at this point that genetic differences in mite populations, in addition to differences in physical environments, could have had an influence on the results obtained. For example, spider mite infestations in Florida can originate from local mite populations or may originate from

Fig. 4. Plot of the first two principal axes from a principal coordinate analysis of a set of *Fragaria* clones in six environments, in cycle H1. Part of the minimum spanning tree is superimposed on the plot, the distances between clones (e.g., 'Totem' - X-11 = 0.50) being the dissimilarities.

Table 4. Lengths of the minimum spanning tree (MST) for the number of spider mites per leaflet².

Clone	MEDLI	MEDHI	MEDTO
A-16	0.462	0.457	0.453
CL-5	0.496	0.448	0.456
E-15	0.473	0.478	0.476
G-9	0.447	0.431	0.437
GCL-8	0.350	0.479	0.411
M-1	0.478	0.445	0.453
RCP-37	0.477	0.429	0.442
TOTEM	0.306	0.407	0.386
WSU 2198	0.304	0.476	0.386
WSU 2202	0.485	0.412	0.453
X-11	0.493	0.500	0.496

²Data are based on eleven *Fragaria* clones grown in six environments. MEDLI = length mean of the MST including only the lowest environments. MEDHI = length mean of the MST including only the highest environments. MEDTO = length mean of the MST including all environments. Clones with values in bold type are consistently represented by points farthest from the center of the scattergram in each cycle (LI, HI, and LI + HI).

populations imported on daughter plants. This may have contributed to the big differences observed between years at Dover, Fla.

Shanks et al. (1995) found that all of the clones in their study supported much fewer mites than did 'Totem' in all six environments. There was, however, a significant genotype $\times$ environment interaction, indicating that some of the resistant clones may be more resistant in certain environments than in others. The present study takes that finding further. The first AMMI analysis indicated that 'Totem' and FL94 were very different from the other entities. They were therefore deleted from a subsequent analysis in order to allow for better separation among resistant clones. This second analysis showed that X-11, CL-5, and M-1 were the most

stable, and X-11 and CL-5 were not only stable, but supported the fewest mites.

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