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Genotypic and Phenotypic Correlations for Reaction to Five Foliar Pathogens in Alfalfa

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Summary. Genotypic and phenotypic correlations for reaction to five foliar pathogens were estimated from analyses of variance and covariance in eight, five-parent diallel crosses each in Saranac, MSA-C4, and MSB-C4 alfalfa (*Medicago sativa* L.). The foliar pathogens were *Uromyces striatus*, Schroet. var. *medicaginis* (Pass.) Arth., *Stemphylium botryosum* Wallr., *Pseudopeziza medicaginis* (Lib.) Sacc., *Phoma herbarum* West var. *medicaginis* Fckl., and *Leptosphaerulina briosiana* (Poll.) Graham and Luttrell.

Genotypic correlations for reaction to *U. striatus* and *P. medicaginis* were significant and positive in Saranac and MSB-C4, but were significant and negative in MSA-C4. Genotypic correlations involving *L. briosiana*, *S. botryosum*, and *P. medicaginis* were positive and significant in MSA-C4 and MSB-C4, but were not significant in Saranac. Significant positive genotypic correlations were observed for reaction to *U. striatus* and *P. herbarum*, and to *P. herbarum* and *S. botryosum* in Saranac and for *U. striatus* and *L. briosiana* in MSA-C4. Correlated responses in selection experiments involving the above, or closely related cultivars, confirmed most of the significant genotypic correlations. Phenotypic correlations were generally much smaller than the genotypic correlations.

Introduction

A knowledge of the genotypic and phenotypic relationships between characters in alfalfa, *Medicago sativa* L., is useful in planning breeding programs. Two examples will illustrate the point: (1) Theory indicates that the greatest response to selection for several traits would be realized by using a selection index (Hazel and Lush, 1942; Smith, 1936). The genotypic and phenotypic variances and covariances must be known before a selection index can be constructed. (2) Breeding programs have been successful in improving resistance to a number of alfalfa pests (Hanson *et al.*, 1972). Genetic variation for resistance is usually present after improving the population, because selection in alfalfa seldom fixes alleles for resistance. Thus, the potential for undesirable shifts through correlated responses in subsequent selection programs remains a possibility. A knowledge of the interrelationships among traits gives some idea of the magnitude and direction of the shifts, and steps can be taken to minimize undesirable shifts.

This paper reports estimates of genotypic and phenotypic correlations for reaction to five foliar pathogens in three alfalfa cultivars.

Materials and Methods

Eight sets of five randomly selected plants from each of three alfalfa cultivars (Saranac [Murphy and Lowe, 1966], MSA-C4, and MSB-C4 [Hanson *et al.*, 1972]) were crossed in diallel combinations within sets during the winter of 1969–70. Air suction emasculation was used to reduce the amount of self-pollination. Four replications of eight plants of each cross were established in flats in the greenhouse in the fall of 1970. The plants were spaced on approximately 3-cm centers in the flats. Replications were randomized within sets in each cultivar, and a replication consisted of one flat of the 10 crosses in a diallel. The crosses in each cultivar were evaluated in simultaneous, but separate experiments. Procedures used by Hill, Leath, and Zeiders (1972) were followed in inoculating the plants with *Uromyces striatus* Schroet. var. *medicaginis* (Pass.) Arth. (rust), *Stemphylium botryosum* Wallr. (Stemphylium leafspot), *Pseudopeziza medicaginis* (Lib.) Sacc. (common leafspot), *Phoma herbarum* West. var. *medicaginis* Fckl. (Ascochyta leafspot or blackstem), and *Leptosphaerulina briosiana* (Poll.) Graham and Luttrell (Leptosphaerulina leafspot or pepper spot) during the winter of 1970–71. Plants were rated 1 = resistant (no lesions or small flecks), 2 = intermediate (small lesions), and 3 = susceptible (large lesions) for reaction to each of the pathogens. Additional details on experimental procedures were given with a report of genetic variances and heritabilities for reaction to the above pathogens in the above cultivars (Hill and Leath, 1972).

Genotypic and phenotypic correlations were estimated from analyses of variance and covariance. Plants without a recorded score for one or more pathogens were eliminated from the analyses of variance and covariance. The genotypic correlation for each pair of pathogen scores was estimated as:

$$r_g = s_{xy_g} / (s^2_{x_g} \cdot s^2_{y_g})^{1/2}$$

where $s^2_{x_g}$, $s^2_{y_g}$, and s_{xy_g} represent estimates of the general combining ability variance and covariance, respectively, pooled over sets within a cultivar for traits x and y.

Estimates of the phenotypic correlation coefficients were computed as:

$$r_p = s_{xy_p} / (s^2_{x_p} \cdot s^2_{y_p})^{1/2}$$

where $s_{xy_p} = s_{xy_w} + s_{xy_e} + s_{xy_s} + s_{xy_g}$, with sub-subscripts w, e, s, and g representing plants in plots, replications by crosses error, specific combining ability, and general combining ability, respectively. A similar calcu-

lation was performed for $s^2_{x_p}$ and $s^2_{y_p}$, except variances instead of covariances were used. Degrees of freedom for plants in plots were 1580, 1531, and 1383 for Saranac, MSA-C4, and MSB-C4, respectively, and those for error, specific combining ability, and general combining ability were 216, 40, and 32, respectively in each cultivar.

The correlation coefficient for plants in plots was computed as:

$$r_w = mp_w/(msx_w \cdot msy_w)^{1/2}$$

where mp_w, msx_w, and msy_w represent the mean product and mean squares from the analysis of variance and covariance, respectively. Standard statistical tables were used to test the significance of the correlations for plants in plots.

Significance of the genotypic and phenotypic correlations was determined by a comparison of each correlation wits its standard error. The standard error was computed as the square root of the variance of the estimated correlation coefficient, and the variance of each correlation was computed according to procedures given by Mode and Robinson (1959). Only those correlations which exceeded three times their standard error were judged significantly different from zero. This decision was based on Fisher (1936, Chap. VI), who indicated that application of the usual rule of concluding significance when correlations exceed twice their standard error results in an inflated Type-I error rate. A computer simulation study by VanVleck and Henderson (1961) indicated that procedures similar to those of Mode and Robinson (1959) tend to under-estimate the variance of genotypic correlations, especially when sample size and heritability are small. Thus, data from selection experiments involving the cultivars included in this study were examined for agreement with the estimated genotypic correlations.

Results

Genotypic correlations for reaction to *U. striatus* and *P. medicaginis* exceeded three times their standard errors in each cultivar (Table 1). The correlations were positive in Saranac and MSB-C4 and negative in MSA-C4, indicating that linkage was probably involved. Had the genotypic correlation been caused by a common resistance factor, correlations of the same sign would have been expected in each cultivar. The cultivars had different origins, and different linkage patterns could have been developed during synthesis of the cultivars. Linkage equilibrium probably would not have been reached between the initial and the certified-seed generation in Saranac, and the selection histories of MSA-C4 and MSB-C4 (Hanson *et al.*, 1972) could have easily hampered the approach to linkage equilibrium.

The validity of the estimated genotype correlations for reaction to *U. striatus* and *P. medicaginis* in MSA-C4 and MSB-C4 can be checked by examination of the selection histories of these cultivars. MSA-C4 and MSB-C4 were derived by three cycles of selection for resistance to *P. medicaginis* in MSA-L4 and MSB-L4, respectively (Hanson *et al.*, 1972). The four cultivars were tested for disease reaction in a single experiment (Hill, Hanson, and Busbice, 1969). Score means for reaction to *U. striatus* were 1.8 in MSA-L4 and 2.6 in MSA-C4, indicating a significant decrease in resistance, and confirming the significant negative correlation for reaction to *U. striatus* and *P. medicaginis* in MSA-C4. *U. striatus* score means in MSB-L4 and MSB-C4 were 1.7 and 1.8, respectively, which was not a significant difference. The increase in resistance to *U. striatus* with selection for resistance to *P. medicaginis*, as indicated by the significant positive genotypic correlation, did not occur. However, MSB-C4 and MSB-L4 were highly resistent to *U. striatus* and a further increase

Table 1. *Estimates of genotypic correlation coefficients and their standard errors for the indicated pathogen scores in three alfalfa cultivars. General combining ability variances are given in parentheses*

Cultivar and pathogen	Pathogen				
	U. striatus	*P. herbarum*	*L. briosiana*	*S. botryosum*	*P. medicaginis*
Saranac					
U. striatus	(0.0110)	0.085 ± 0.025*	0.792 ± 0.438	−0.558 ± 0.214	0.213 ± 0.047*
P. herbarum		(0.0027)	0.566 ± 0.356	0.365 ± 0.099*	0.611 ± 0.272
L. briosiana			(0.0039)	−0.578 ± 0.313	0.261 ± 0.211
S. botryosum				(0.0026)	0.160 ± 0.103
P. medicaginis					(0.0104)
MSA-C4					
U. striatus	(0.0337)	−0.083 ± 0.119	0.177 ± 0.018*	0.279 ± 0.159	−0.154 ± 0.017*
P. herbarum		(0.0009)	0.685 ± 0.791	2.076 ± 2.761	0.632 ± 0.694
L. briosiana			(0.0133)	0.230 ± 0.063*	0.357 ± 0.006*
S. botryosum				(0.0050)	0.520 ± 0.134*
P. medicaginis					(0.0092)
MSB-C4					
U. striatus	(0.0368)	−0.556 ± 0.204	0.462 ± 0.193	−0.296 ± 0.125	0.328 ± 0.045*
P. herbarum		(0.0040)	−0.939 ± 0.600	−0.597 ± 0.363	−1.205 ± 0.441
L. briosiana			(0.0097)	0.278 ± 0.097	0.457 ± 0.116*
S. botryosum				(0.0036)	0.630 ± 0.118*
P. medicaginis					(0.0158)

* indicates correlations that exceed three times their standard errors.

Table 2. *Estimates of phenotypic correlations and their standard errors (top figure) and of simple correlations for plants in plots (bottom figure) for the indicated pathogen scores in three alfalfa cultivars. The respective variances are given in parentheses*

Cultivar and pathogen	Pathogen				
	U. striatus	*P. herbarum*	*L. briosiana*	*S. botryosum*	*P. medicaginis*
Saranac					
U. striatus	(0.2559) (0.2330)	0.055 ± 0.001 0.050	0.082 ± 0.002 0.113*	0.014 ± 0.001 0.015	0.048 ± 0.001 0.031
P. herbarum		(0.1660) (0.1434)	0.028 ± 0.002 0.004	0.044 ± 0.001 0.027	−0.001 ± 0.000+ −0.040
L. briosiana			(0.4724) (0.4515)	0.043 ± 0.001 0.054	0.032 ± 0.001 0.030
S. botryosum				(0.2004) (0.1900)	0.018 ± 0.001 0.002
P. medicaginis					(0.4012) (0.3573)
MAS-C4					
U. striatus	(0.3835) (0.2812)	0.024 ± 0.001 −0.013	0.086 ± 0.003 0.080**	0.037 ± 0.001 0.067*	−0.002 ± 0.000+ −0.002
P. herbarum		(0.1368) (0.1201)	−0.011 ± 0.000+ −0.021	−0.004 ± 0.000+ 0.030	−0.053 ± 0.001 −0.045
L. briosiana			(0.2722) (0.2496)	0.043 ± 0.001 0.051	0.153 ± 0.003 0.129**
S. botryosum				(0.2185) (0.1874)	0.032 ± 0.001 0.023
P. medicaginis					(0.2014) (0.1802)
MSB-C4					
U. striatus	(0.4907) (0.3991)	−0.044 ± 0.001 −0.013	0.013 ± 0.000+ 0.041	0.044 ± 0.001 0.027	0.063 ± 0.002 0.030
P. herbarum		(0.1794) (0.1379)	0.010 ± 0.000+ −0.015	−0.024 ± 0.000+ −0.046	0.001 ± 0.000+ 0.099**
L. briosiana			(0.2827) (0.2363)	0.021 ± 0.000+ 0.004	0.130 ± 0.003 0.134**
S. botryosum				(0.1246) (0.1113)	0.077 ± 0.001 0.077**
P. medicaginis					(0.2201) (0.1987)

* and ** indicate correlation coefficients significantly different from zero at the 0.05 and 0.01 probability levels, respectively.

in resistance would have been difficult with selection for *U. striatus* resistance alone. Thus, the results of Hill *et al.* (1969) do not conflict with the estimated genotypic correlation in MSB-C4.

A pattern associated with cultivars was observed with estimates of the genotypic correlations involving *L. briosiana*, *S. botryosum* and *P. medicaginis*. No correlation in this group exceeded three times its standard error in Saranac, but each one in this group (except the correlation for reaction to *L. briosiana* and *S. botryosum* in MSB-C4) exceeded three times its standard error in MSA-C4 and MSB-C4 (Table 1). All the correlations involving the above pathogens in MSA-C4 and MSB-C4 were positive, indicating that plants genetically resistant to one of these pathogens tended to be resistant to the other two. The correlations in MSA-C4 and MSB-C4 were similar in magnitude for each pair of scores.

The genotypic correlations for reaction to *U. striatus* and *P. herbarum* and to *P. herbarum* and *S. botryosum* in Saranac exceeded three times their standard errors (Table 1). Neither of these correlations were significant in MSA-C4 or MSB-C4. Haag and Hill (1974) observed correlated responses to selection that confirm the significance of both these correlations: polycross progeny test and polycross family selection for resistance to *U. striatus* in an experimental population derived from Saranac resulted in a significant increase in resistance to *P. herbarum*; selfed progeny test selection for resistance to *P. herbarum* resulted in a significant increase in resistance to *U. striatus*; individual selection for resistance to *S. botryosum* resulted in a significant increase in resistance to *P. herbarum*.

The only remaining genotypic correlation that exceeded three times its standard error was for reaction to *U. striatus* and *L. briosiana* in MSA-C4 (Table 1). This correlation did not exceed three times its standard error in Saranac or MSB-C4. Supporting evidence from previous selection experiments in the parent populations of MSA-C4 was not available.

Phenotypic correlations were usually much smaller than their respective genotypic correlations (Table 2). Searle (1961) demonstrated that phenotypic correlations could be much smaller than genotypic correlations when heritability was low. The predominant component of variance in our experiments was that due to plants in plots, which results in low heritability (Hill and Leath, 1972), and the plants in plots component of variance was the main cause of the difference in magnitude of the genotypic and phenotypic correlations.

Each of the phenotypic correlations exceeded three times its standard error (Table 2), suggesting that the standard errors were probably underestimated by the procedure used. The phenotypic correlations were similar in magnitude to the correlation coefficients for plants in plots. We concluded that the phenotypic correlations were relatively unimportant unless accompanied by a significant correlation for plants in plots. The only significant correlation for plants in plots was for reaction to *U. striatus* and *L. briosiana* in Saranac and MSA-C4, *U. striatus* and *S. botryosum* in MSA-C4, *P. herbarum* and *P. medicaginis* in MSB-C4, *L. briosiana* and *P. medicaginis* in MSA-C4 and MSB-C4, and *S. botryosum* and *P. medicaginis* in MSB-C4. All of these, as well as most of the other correlations, were positive. This indicates that plants resistant to one of the pathogens had a tendency to be phenotypically resistant to the others, but the tendency was very weak.

Discussion

We followed recommendations of Dudley, Busbice and Levings (1969) in using several small diallel crosses in a pooled analysis instead of a larger partial diallel. Sampling variation among diallel crosses with only five parents may be quite large, however, and this variation could lead to biased estimates of variances, covariances, and correlations. This illustrates one of the dilemma in obtaining quantitative genetic information on many crop species in which controlled crossing may be difficult; few breeding projects would have the resources to evaluate a much larger number of crosses than were evaluated in our experiment. Data from such modest sized experiments as ours should be interpreted with extreme caution.

Van Vleck and Henderson (1961) concluded that at least 1000 sets of observations are needed to obtain reasonable estimates of the sampling variances of genotypic correlations, and that accurate estimates may be impossible when heritability is low. Our results support their conclusion that estimation is more difficult when heritability is low — correlations involving *P. herbarum*, *L. briosiana*, and *S. botryosum*, for which heritability of resistance is low (Hill and Leath, 1972), were very variable and had large standard errors. However, the fact that most of our significant correlations could be verified by correlated responses in selection experiments suggests that the recommendations of Van Vleck and Henderson (1961) on sample size may not strictly apply to analysis of disease score data on alfalfa.

The variability inherent in estimates of genotypic correlations indicates that interpretation of them is practically impossible without estimates of their standard errors. Estimates of genotypic correlation indicate general trends, and are so variable that accurate and meaningful estimates of correlated response to selection or selection index weights have little value. Even if accurate estimates were obtainable, the differences between cultivars indicate that separate estimates would be needed for each population. In a theoretical study of the selection index, Williams (1962) concluded that estimates of the weights were highly variable, and suggested use of a base index. Elgin *et al.* (1970) found that the base index was at least as good as the estimated index for disease resistance in alfalfa.

The majority of the genotypic correlations, and all but one of the significant correlations, were positive. This is a fortunate circumstance for alfalfa breeders, because it indicates that selection for resistance to one pathogen will generally not decrease resistance to others. The fact that significant positive correlations were more frequent in MSA-C4 and MSB-C4 than in Saranac further suggests that the frequency of plants containing some form of a common resistance mechanism may have been increased during selection for disease resistance in these cultivars. Such a mechanism might be (*1*) increased amounts of some chemical constituent present in the plant prior to infection, (*2*) an induciable phytoalexin, such as medicarpin, which is produced in alfalfa leaves infected by *P. herbarum*, *L. briosiana*, or *S. botryosum* (Higgins, 1972); or (*3*) the more rapid accumulation of necrogenous compounds having nonspecific, antifungal activity. Any of these hypotheses would explain the pattern of genotypic correlations observed in MSA-C4 and MSB-C4, as well as the fact that 21 of the 30 genotypic correlations were positive.

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