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A laboratory and field survey of leaf feeding resistance in diverse maize inbred lines

--Forde, AJ; Applewhite, HS; Bass, HW

Using a common garden plot in Northern Florida (Mission Road Field, FSU) that was established for an unrelated genetic study, we assessed natural levels of insect damage exhibited by a highly diverse population of inbred maize lines (Liu et. al., Genetics 165:2117, 2003). The field contained families for seed propagation from two sets of maize diversity lines developed by E. S. Buckler, J. Holland, M. McMullen and Goodman. The two sets of lines grown in 2008 are referred to by us as the "MDS" set (260 of the 283 lines from Order 185771, Feb 2007) and the "DL25" set (25 of the 26 lines from Order 179535, Apr 2006) originally provided by the USDA, ARS, NCRPIS, ISU, Ames, IA. The MDS lines were planted in families of 16, 12, or 8 plants each and the DL25 lines were planted in two different locations within the same field in families of 24 or 14 plants each.

Cursory inspection of insect damage prior to pesticide treatment revealed potential line-dependent variation in herbivory damage, primarily from lepidoptera exposure. To investigate the possible genotypic basis for this observed variation, we measured field damage in the DL25 lines and carried out laboratory bioassays on a selected subset of them. The DL25 lines, plus a common-parent, B73, have been used to develop a nested association mapping (NAM) population (Yu et al., Genetics 178:539). A general objective of the study described here was to determine which lines and corresponding NAM recombinant inbred lines might be useful for subsequent genetic analyses of complex quantitative traits related to maize-lepidoptera interactions.

Data was collected from our DL25 set, B73, B97, CM103, CML228, CML247, CML277, CML322, CML333, CML52, HP301, Il14H, Ki11, Ki3, Ky21, M162W, M37W, Mo17, Mo18W, Ms71, NC350, NC358, Oh43, Oh7B, P39G-B, Tx303, and Tzi 8. Insect surveys revealed that the damage present on plants in our plot was caused primarily by two species of caterpillars, *Spodoptera frugiperda* (J. E. Smith), the fall armyworm, and *Spodoptera ornithogalli* (Guenée), the yellowstriped armyworm. The amount of damage sustained by plants was quantified using a rating scale of 0-4, with larger ratings indicating that damage was present on a greater proportion of leaves from an individual plant.

Leaf damage varied significantly among lines according to analysis of covariance as shown in Figure 1 (F=5.48, df=25, P<0.001; using MIXED procedure; SAS version 9.2; SAS Institute, 2008). Additionally, planned contrasts found that five lines were significantly more damaged and five were significantly less damaged compared to the population average (Fig. 1). A spatial covariance matrix was incorporated into the ANCOVA to account for autocorrelation because, within each of the two blocks in the plot, all individuals of a maize line were planted in "family groupings" located along contiguous stretches within rows.

Induced and constitutive antibiosis resistance laboratory phenotype data was collected from herbivore growth rate bioassays for nine (B73, CML52, HP301, IL14H, KY21, M162W, M37W, NC350, Tzi 8) of the 26 maize lines present in the field plot, plus
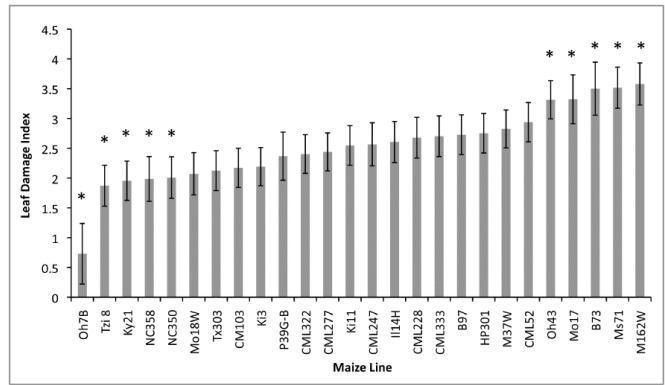

Figure 1. Mean damage index values (±SE) assessed for maize plants (mid to late whorl/V8/8-leaf-collar stage) from different inbred lines in a field in Northern Florida. Damage ratings were assessed visually, and were based on the percentage of leaves exhibiting any chewing damage. The percentage equivalent of each damage index value are as follows: a rating of 0 corresponds to 0-10%, 1 to 11-25%, 2 to 26-50%, 3 to 51-75% and 4 to 76-100%. Asterisks denote lines demonstrating levels of damage that were significantly different when compared to the average amount of damage for all other lines (according to planned contrasts, P<0.05). Means represent least square means produced from an ANCOVA model incorporating plant size and spatial location as covariates. Average sample size was 23.92.

Sorghum bicolor and teosinte (Balsas, *Zea mays* ssp. *parviglumis*). The subset of nine from the DL25 lines was chosen in order to encompass as much phenotypic and genetic variation as possible, using our field damage estimates and knowledge of phylogenetic relationships. Second instar fall armyworm caterpillars were fed foliage from herbivore-damaged or undamaged plants (V4/4-leaf-collar stage) grown under greenhouse conditions, and their weight gain over a 48-hour period was measured. An ANOVA on caterpillar growth rates revealed significant variation in constitutive resistance among the lines, as shown in Figure 2 (Main effect of line: F=2.74, df=10, P=0.0028), but provided no obvious evidence of induced resistance (Main effect of leaf damage: F=0.09, df=1, P=0.76; Damage x Line interaction F=1.21, df=10,P=0.33). According to multiple comparisons, caterpillars grew significantly slower when fed NC350, compared to all other lines except Tzi 8. Four of these differences remained significant after a highly conservative Bonferroni p-value correction (Fig. 2). Furthermore,
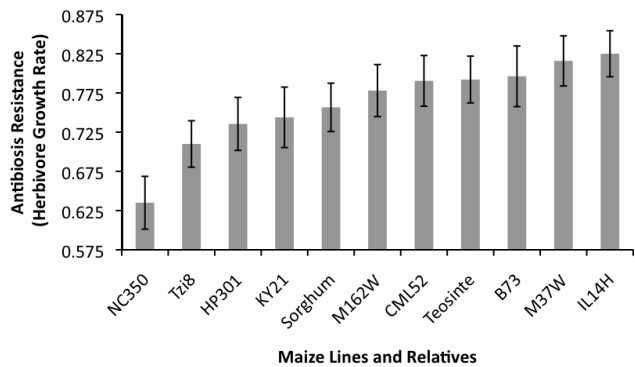

Figure 2. Constitutive antibiosis resistance, assayed by relative growth rates (ln(final mass) – ln(initial mass)) of fall armyworm caterpillars (±SE) fed leaves from different greenhouse/lab-grown maize lines. Faster growth rates denote less resistance. Each caterpillar was allowed to feed on the newest expanded leaf of a plant (excised) for 48 h in a no-choice situation. Plants had been grown in a greenhouse under standard conditions and were stage V4 (4 leaf-collar stage). Means represent least squared means produced from an ANOVA model incorporating a main effect of line. Significant differences were found between NC350 and four lines (B73, CML52, Il14H, M37W) when p-values of multiple comparisons were adjusted with a Bonferroni correction. Average sample size was 39.9.

levels of constitutive resistance in the laboratory were significantly related to the amount of damage that was sustained by lines in the field, as shown in Figure 3 (Likelihood ratio test: deviance=1.267, df=7, P=0.033).
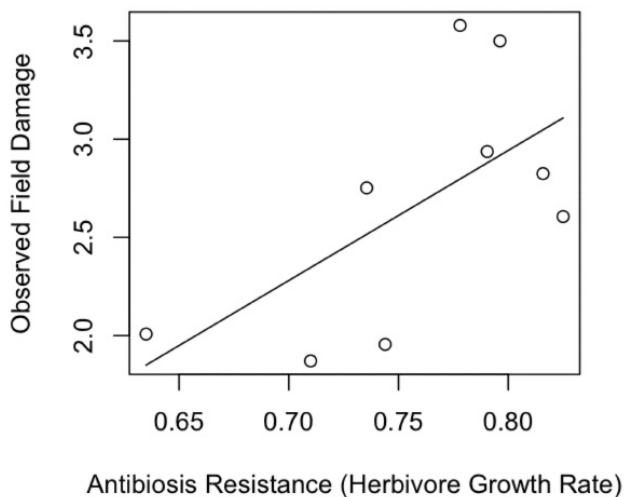

Figure 3. The relationship between average damage index values and average antibiosis resistance (as measured by herbivore growth rates) for the chosen subset of nine lines. Antibiosis resistance demonstrated a significant linear relationship with observed damage levels according to a likelihood ratio test (deviance=1.267, df=7, P=0.033).

The concordance between the patterns of resistance observed in the field versus laboratory suggests that constitutive antibiosis resistance expressed by the lines is important for deterring leaf damage. To the extent that this relationship holds up, these types of herbivore growth rate bioassays may provide an efficient method to pre-screen germplasm for resistance prior to more extensive field trials. Despite the fact that our observations of damage in the field were predicted by laboratory measures of resistance, follow-up studies will still be needed in order to confirm whether levels of resistance in these lines are stably expressed across seasons, locations, and developmental stages. This study confirms our suspicion that these maize diversity lines could be used to learn more about the genetic basis of herbivore resistance and the effect of genetic variation in plant defense on ecological dynamics.

URBANA, ILLINOIS
Maize Genetics Cooperation • Stock Center

Allelism testing of miscellaneous stocks in the Maize COOP phenotype only collection
--Jackson, JD; Harper, C

This report summarizes allele testing of miscellaneous stocks characterized by phenotype only in the Maize Genetics COOP Stock Center collection. Crosses were made between known heterozygotes if possible. Ears were shelled and planted in sand benches to score seedlings for the appropriate phenotypes. Plants from the lazy crosses were scored in the field at maturity. Proposed new designations have been assigned to these alleles. These stocks have been increased and placed on our stocklist. It is expected that with further sorting and allelism testing of mutations characterized by phenotype only, additional alleles of characterized mutants will be discovered and placed in the main collection.

POSITIVE TESTS:

previous designation	allelism test with *spt1*	new designation	MGCSC: stock number
spt-92-3239-53	positive: (+ / *spt1-N464*) x (+ / *spt*)	*spt1-92-3239-53*	226J

previous designation	allelism test with *oro1*	new designation	MGCSC: stock number
oro-85-3087-3	positive: (+ / *oro1-6474*) x (+ / *oro*)	*oro1-85-3087-3*	616C
oro-88-89-3550-32	positive: (+ / *oro1-6474*) x (+ / *oro*)	*oro1-88-89-3550-32*	616D

previous designation	allelism test with *la1*	new designation	MGCSC: stock number
la-05HI-RnjxW22GN-333	positive: (+/*la1*) x *la*	*la1-05HI-RnjxW22GN-333*	406E
la-MTM4659	positive: (+/*la1*) x *la*	*la1-MTM4659*	406F

New alleles of *chlorophyll1* found in lemon white endosperm stocks in the Maize COOP phenotype-only collection
--Jackson, JD

This report summarizes allele testing of lemon-white endosperm stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here pale kernels linked to pale-green or albino seedlings characterized all stocks. Many had previously given negative results in tests with *vp9*, *w3* and *y9*. The *cl1 Clm1-3* stock used in crosses here carries a dominant modifier of *cl1* that allows for viable green plants, making crosses with a homozygous stock possible. Crosses were made as follows: [+//*lw*]@ X *cl1 Clm1-3* or +//+//*lw* X *cl1 Clm1-3*. Ears were scored for the segregation of pale yellow kernels.

New designations have been assigned to these alleles and they have been placed in the main collection. Stocks with this same phenotype that were found to complement *cl1 Clm3* will be tested for allelism with other stocks linked to pale endosperm.

Previous designation	allelism test with *w3*	New designation	MGCS stock number
5705F *pale-y*-87-88-2679-1	4 positive	*cl1-87-88-2679-1*	306H
5908Q *y-vp*-1982-1	3 positive	*cl1-1982-1*	306I
5910M *pale-y*-85-3007-40	3 positive	*cl1-85-3007-40*	306J
5912P *lw-y-pg*-1998-4	5 positive	*cl1-1998-4*	306K

New alleles of *white3* found in viviparous stocks in the Maize COOP phenotype only collection
--Jackson, JD

This report summarizes allele testing of various viviparous and lemon-white endosperm stocks characterized only by phenotype in the Maize Genetics COOP Stock Center collection. Here pale kernels linked to pale or albino seedlings characterized all stocks. Many had previously given negative results in tests with *vp9* and *y9*. The *w3-y11* stock used in crosses here is homozygous viable. Crosses were made as follows: [+/*vp*]@ X *w3-y11* and +//+/*vp* X *w3-y11*. Ears were scored for the segregation of pale yellow kernels. In most cases, pale-yellow kernels were selected from positive allele test ears and planted in the field for observation. Seed-