Among the mutants, 43 out of 60 showed total resistance to head smut. Fourteen of the mutants, however, had a frequency of infection surpassing the standard value significantly, from 10 to 44 per cent.

Thirty-six lines were fully resistant to common smut in the provocation experiment. Of these, 28 lines were totally resistant in the provocation experiment for head smut; therefore, in 28 mutants the two kinds of resistance were to be found together. Some mutants, however, in the control experiment showed a much higher susceptibility for common smut as well (10-33%). As for Fusarium, in two experiment places 14 mutants were fully resistant, and 7 mutants were fully resistant to the two smut and the Fusarium damages together.

The investigations will be repeated in 1968. The genetical analysis of resistance in the stably resistant form will be begun after this control only.

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1. Lysine microbiological assay.

A procedure has been developed using a microbiological assay to rapidly and accurately determine the lysine content of corn grain samples. Samples are prepared for analysis by hydrolyzing a measured scoopful (approximately 200 mg) of ground corn in 10 ml of 6 N HCl at 110°C for 24 hours. Hydrolysis is carried out in a 10 ml screw cap vial. The caps are fitted with Teflon liners and care is taken to insure that the caps fit tightly. After hydrolysis the sample is filtered into a 50 ml beaker and evaporated on a hot plate to dryness. The samples are stored dry in a freezer until ready for assay. Just prior to assay the samples are resuspended in 25 ml of 0.05 M phosphate buffer pH 6.8.

The assay itself is a modified version of the one described by Difco Laboratories. The organism used is Leuconostoc mesenteroides and all procedures are identical except that a 3 ml assay volume is used instead of 10 ml. This results in a considerable saving of media and the assay is read directly in the culture tubes. Growth is determined by increase in turbidity at 660 mu.

Nitrogen is determined on an aliquot of the hydrolyzed sample by Nessler's procedure. Nitrogen determination on an aliquot of the same sample as is used for the lysine assay permits direct expression of lysine in terms of the nitrogen content of the sample and also does not require that gravimetric procedures be used in sample preparation, i.e., the sample does not have to be weighed and the filter paper need not be washed quite so thoroughly .

Typical results are shown in the table below for whole kernel.

Pedigree	Grams Lysine / 100 grams Protein	± 1 Standard deviation
R109B +/+	3.02	± .35
R109B o_2/o_2	5.42	± .41

Work is also progressing in our lab for a single kernel assay.

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