Compositional Analysis Phase Report

Final Report Title

Compositional Analysis of Maize MPS Hybrid Line 1507

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Phase Completed On

July 18, 2000

Performing Laboratories

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Study Number

98-09-RA-NGLP-012

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Microbial Cry1F (truncated) Delta-Endotoxin

Title: Compositional Analysis of Maize MPS Hybrid Line 1507

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C).*

Company: Dow AgroSciences LLC

Company Agent: P.L. Hunst

Title: Regulatory Manager

Signature: Penny L. Hunst

Date: 11/29/00

*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE

At the time this study was conducted, it was not subject to Good Laboratory Practice Standards and was, therefore, not monitored by the quality assurance unit.

Rod Townsend, Study Sponsor — 07-26-00 — Date

Cynthia Stauffer, Study Director — 18 July 2000 — Date

Study Number: 98-09-RA-NGLP-012

Title: Compositional Analysis of Maize MPS Hybrid Line 1507.

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Graneros, Chile.
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Buin, Chile.

Study Sponsor: Rod Townsend, Pioneer Hi-Bred Int'l Inc.

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- Jan Hazebroek (tocopherols) Pioneer Hi-Bred International, Inc. Analytical Biochemistry Laboratory.

Study Initiation Date: 10/12/98

Records Retention: All study specific raw data, protocols, final reports and facility records are archived initially at Pioneer Hi-Bred Int'l Inc. and will be retained permanently at QAI in Columbia, MD.

Specimen Storage: All retain samples will be stored at Pioneer in Johnston, IA.

I certify that this report accurately represents the results observed during the course of this study.

Report issued by:

Cynthia Stauffer, Study Director — 18 July 2000
Date

Rod Townsend, Study Sponsor — 07-26-00
Date

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ABSTRACT

Maize line 1507 was modified to express the Cry1F protein from *Bacillus thuringiensis* subsp. *aizawai*. This protein confers resistance to the European Corn Borer (*Ostrinia nubilalis* Hubner) insect pest. This line also contains a synthetic *pat* gene, derived from *Streptomyces viridochromogenes*, which encodes phosphinothricin acetyl transferase (PAT). The PAT protein is an enzyme that inactivates the herbicide glufosinate-ammonium and thus makes genetically modified plants that accumulate this protein tolerant to the herbicide.

The purpose of the field trial was to measure levels of various nutritional composition traits in grain and whole plant tissue samples. This report summarizes the composition analysis phase of this study.

The test system for this study consisted of four field sites located in the major maize growing regions of Chile. The sites were between 10 and 125 km south of Santiago. The test system represents locations equivalent to regions in the United States where the maize lines would be suitable commercial products, therefore the nutritional composition traits in grain and whole plant tissue samples should be equivalent to what can be expected in the United States. At each site, grain samples for compositional analysis consisted of grain from three ears pooled together. The whole plant samples consisted of three plants pooled together.

The results of the nutritional composition analysis of grain and forage from *B.t.* Cry1F maize line 1507 show that it is comparable to commodity corn.

I. INTRODUCTION

A. Background

B.t. Cry1F maize line 1507 has been modified with the *cry*1F gene from *Bacillus thuringiensis* that encodes the Cry1F protein. The Cry1F protein possesses insecticidal activity against the European corn borer (ECB) pest. The ECB resistant test substance line also contains a synthetic *pat* gene, derived from *Streptomyces viridochromogenes,* which encodes the PAT protein (enzyme) that confers tolerance to the herbicide glufosinate-ammonium. The PAT protein is present to enable the selection of cells, in tissue culture, that contain the *cry*1F gene and also confers tolerance to glufosinate-ammonium under field conditions. The control lines have the background genetics representative of the test substance line but are not genetically modified and therefore, do not express the Cry1F or PAT proteins.

B. Purpose

The purpose of this study was to generate data on protein expression levels in the test substance line and nutritional composition data on grain and whole plant forage from both the test and control substance lines. All tissue samples obtained from the inbred and hybrid lines were evaluated for expression levels of the Cry1F and PAT proteins. Grain and whole plant samples of only the hybrid test and control lines were evaluated for composition.

This report, titled Compositional Analysis of Maize MPS Hybrid line 1507, summarizes the composition analysis phase of study 98-09-RA-NGLP-012. It is a companion report to study report 98-09-RA-NGLP-012, which summarized the protein expression analyses and was completed on December 1, 1999. The data generated on key nutrient and anti-nutrients in both the test substance and control lines will be used in food and feed safety evaluations of *B.t.* Cry1F maize line 1507.

II. MATERIALS

A. Test substance

The test substance for the compositional phase of this study was seed of a maize line 1507. Plants grown from this seed are capable of expressing the Cry1F and PAT proteins.

Initial characterization of the test substance consisted of documentation of the breeding lineage of the seed. Pedigree information for the hybrid and inbred is proprietary information and is on file with staff breeders at Mycogen Seeds, Huxley, Iowa. Prior to planting, the seed was stored under appropriate conditions to maintain seed viability and vigor (Wych, 1988). Definitive characterization of the test substance occurred during the study. Confirmation of tolerance to glufosinate-ammonium in the field and specific ELISA's for the detection and quantification of the Cry1F and PAT proteins were performed.

B. Control substance

The control substance for the compositional phase of this study was seed from a maize hybrid that had not been genetically modified, but that had background genetics representative of the test substance (designated Hybrid A_M). Pedigree information for Hybrid A_M is on file with staff breeders at Mycogen Seeds, Huxley, Iowa. Prior to planting, the seed was stored under appropriate conditions to maintain seed viability and vigor (Wych, 1988).

C. Reference substances

Appropriate standards were used in the procedures employed for the analyses of nutrient composition.

D. Test system

The test system for this study was the environment in which the maize plants were grown. The field sites were Buin, Viluco, Graneros and Nancagua, Chile all located between 10 and 125 Km south of Santiago. These sites are located within the major maize growing regions of Chile and are equivalent to regions in the United States where the maize lines would be suitable commercial products. Therefore, the measurements of key nutritional components of maize grain and forage should be comparable to what can be expected in the United States. Each site was identified by a unique 3-character code.

At each site, there were six blocks (block = replicate) that were arranged in a randomized complete block design with nesting. Block 1 consisted of three-row plots for each entry and contained the test and control lines. Blocks 2 through 6 consisted of two-row plots containing the test and control line. Each line was identified in the field by a range/row designation. Field plot maps showing the randomization schemes for each site are shown in Tables 1-4.

III. METHODS

A. Summary of experimental design

The test and control lines were grown at four field sites in Chile. Whole plant forage and grain samples from the hybrid test and control lines were analyzed for nutrient composition.

B. Field trial

All sites were managed so that the identity and integrity of all samples was maintained. Important crop dates (i.e. seed receipt, planting, pollinations and harvest) are listed in Table 5.

1. Agronomic practices

Agricultural practices for growing the test and control plants were typical for producing maize in the regions chosen for this study. Chemical and fertilizer applications were appropriate for each location. Chemical and fertilizer application dates and amounts are summarized in Tables 6-9.

2. Planting

The land at each site went through multiple plowings and cultivations to prepare the soil prior to planting. The test and control lines were planted at a depth of 5 to 7 cm. Between 19 and 23 kernels of each line were planted per row of each plot. The row length at the Buin and Viluco sites was approximately 3.75 meters and the row length at the Graneros and Nancagua sites was approximately 3.55 meters. Rows at all sites were approximately 0.75 meters apart.

The test lines were segregating for the Cry1F and PAT genes. All test lines were sprayed with glufosinate-ammonium herbicide using a hand spray bottle at approximately the V5 to V6 stage of development. Results from the herbicide spraying are summarized in Table 10. In Viluco, the control lines were inadvertently sprayed with glufosinate-ammonium instead of the test lines. All the plants in the control plots died; the test lines that had not been sprayed were then sprayed. Plants that were damaged by the herbicide were assumed to lack the *pat* and *cry1F* genes and were removed from the plots of each test line.

3. Climate

Weather conditions were documented at each site and are summarized in Table 11. Rainfall and supplemental irrigation was sufficient to produce maize typical of the growing area.

4. Sampling

Sampling dates are summarized in Table 12.

Whole plant sampling: Three self-pollinated whole plants were collected from each hybrid test and control plot. The samples were chopped and frozen at the Pioneer facility near Buin. The samples were then dried for 2 days at approximately 62°C and kept at room temperature until shipment to Johnston, IA. Samples were forwarded to the Pioneer Livestock Nutrition Center in Polk City, IA and stored frozen until ground to a fine powder. They were then shipped to Woodson-Tenent Laboratories for analysis.

Grain sampling: Grain samples were harvested by hand from each hybrid test and control plot under normal harvest conditions (after physiological maturity). Ears from three self-pollinated plants were collected from hybrid test and control plots in all replicates except replicate #1, where five ears were collected. The additional ears were collected in replicate #1 in order to provide samples for the protein expression analysis. After drying, the ears were individually shelled and then placed in separate labeled bags. The grain samples were shipped to the analytical principal investigator at ambient temperature. Approximately 1 gram of sample from each of five ears from replicate #1 was removed for ELISA analysis. The grain from three ears was pooled, 15 grams of sample was removed for tocopherol analysis and the remaining sample was shipped to Woodson-Tenent Laboratories for analysis. In each remaining replicate, the grain from three ears was pooled, 15 grams were removed for tocopherol analysis and the remainder shipped to Woodson-Tenent Laboratories.

C. Compositional Analyses

1. Forage

Woodson-Tenent Laboratories, Inc. Des Moines, Iowa conducted the following analyses:

Moisture Analysis
Moisture analysis was conducted according to American Oil Chemist's Society (AOCS) method #Ba 2a-38. Moisture is the actual water content and any material that is volatile under the conditions of the test.

Fat Content
Crude fat content was determined by use of the AOCS method #Ba 3-38. This determines the substances extracted by petroleum ether under the conditions of the method.

Protein Content
Crude protein content was determined by use of the AOCS method #Ba 4d-90. Mixtures of true proteins, composed of amino acids, and non-protein nitrogen make up the crude protein content. Protein is determined by measuring the amount of nitrogen and multiplying by 6.25. The general term "Protein" refers to crude protein that includes both available and unavailable crude protein.

Ash Analysis
Ash analysis was conducted according to AOCS method #Ba 5a-49. This method determines, as ash, the residue remaining after incineration under the conditions of this test.

Fiber Content
Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) content was determined by use of the ANKOM$^{200/220}$ method by Woodson-Tenent Laboratories.

2. Grain

Analyses of moisture, fat, protein, ash and fiber content of grain samples were performed using the methods described for whole plants.

Fatty Acid Composition (% relative)
The percent relative fatty acid composition was determined using the AOCS method #Ce 1e-91.

Amino Acid Profile
The amino acid profile was determined using the method by the Association of Official Analytical Chemists (AOAC) 1990 15th Ed 982.30 D,E,F.

Minerals: Ca, P, Cu, Fe, Mg, Mn, K, Zn.

The calcium levels were determined by AOAC (1990 15th Ed.) method 968.08 with modifications as performed by Woodson-Tenent Laboratories.

Phosphorous was determined by AOAC method 965.17.

Potassium, Copper, Iron, Magnesium, Manganese and Zinc were determined using established AOAC methods as performed by Woodson-Tenent Laboratories.

Vitamins: B1, B2, E, and folic acid
Vitamin B1 - AOAC 942.23 with fluorometric quantitation.
Vitamin B2 - AOAC 970.65 with fluorometric quantitation.
Vitamin E - AOAC 971.30 with HPLC quantitiation.
Folic Acid - AOAC 992.05.

Phytic Acid
Phytic acid levels were measured by methods described in Analytical Biochemistry (1977) 77:536-539.

Trypsin Inhibitor
Trypsin inhibitor levels were measured by AOCS method Ba 12-75.

The following analysis was conducted by the Analytical Biochemistry Laboratory (Jan Hazebroek) at Pioneer Hi-Bred Intl., Inc.

Tocopherols
Tocopherol levels were conducted according to AOAC method 971.30.

D. Control of bias

The test substance was planted in randomly assigned locations within each of the four replicates at each test site. The entire test area was uniformly maintained using practices typical of maize production for each growing area.

E. Data reduction and statistical analysis

The study was conducted at four locations with two treatments each (test and control hybrid) in Chile. At each location, six replicates were arranged in a randomized complete block design with nesting. Replicate 1 consisted of three-row plots for each of the control and the test hybrid and inbred (the inbred test material and control were not included in the compositional analysis). Replicates 2 through 6 consisted of two-row plots for each of the hybrid control and the test lines.

A statistical analysis was carried out to test for a difference between the test hybrid and the control hybrid. The initial mixed model used to describe the data is given by:

response = *location rep(location)* treatment *treatment*location*

where random effects are indicated by italics. This model is referred to as the Full Model.

To determine whether *treatment*location* was a significant source of variation a likelihood ratio test was performed. In many cases *treatment*location* was not significant at $\alpha = 0.30$. In those cases the following model was used:

response = *location rep(location)* treatment

where random effects are indicated by italics. This model is referred to as the Reduced Model.

In the few cases where there was a significant interaction between treatment and location the Full Model described above was used.

The level of significance for all responses was $\alpha = 0.05$.

For whole plant forage data, replicate #1 at the Buin site was not included in the analysis (see section III. F. Protocol amendments and deviations below). For analysis of the grain data, all replicates from the Buin location were included.

Data points from P-Coumaric acid and ferulic acid from the control hybrid grown at the Buin location were determined to be outliers and therefore not included in the statistical analysis.

F. Protocol amendments and deviations

The glufosinate-ammonium herbicide was inadvertently applied to the control entries of one of the six replicates at the Viluco site, but was not applied to the test entries. Therefore, samples were obtained from control hybrid plants from five replicates at this location. The lack of data from one replicate at this location was accounted for in the analysis of the data.

Polymerase Chain Reaction (PCR) analysis was performed on the first replicate of the whole plant sample from the Buin location because the Cry1F expression was below the limit of detection. PCR analysis showed that the whole plant sample from replicate #1 at the Buin location was negative for the *cry1F* gene. Therefore, data from this replicate at the Buin site was not included in the statistical analysis.

IV. RESULTS AND CONCLUSIONS

A. Field Trial

Plant samples were collected from test substance and control hybrids that were representative of commercially grown corn. Therefore, data collected on composition levels were representative of the levels expected in the commercial crop of these corn lines.

B. Composition analyses

The conclusions in this report are primarily derived from a comparison of nutrient levels in the test line to levels in the literature, based on standard approaches in food safety assessment. A report from food experts (IFBC, 1990) states that "In evaluating a genetically modified food, a comparison with its traditional counterpart will be necessary in order to determine whether the significant nutrients in the new food as consumed fall within the range typical of the product. If the new product is found to have essential nutrients in the same range as its traditional counterpart, no further nutritional evaluation of the product would be required." The term "traditional counterpart" is defined as the comparable traditional foods and ingredients. The report states that the general classes of inherent constituents that should be evaluated include key nutrients, naturally occurring toxicants, and constituents that affect the processing of food; however, an exhaustive analytical comparison is not necessary in most cases. This concept, known as substantial equivalence, has been embodied in regulatory policies such as the US Food and Drug Administration policy on new plant varieties (FDA, 1992).

The statistical analysis included means for the test line, *B.t.* Cry1F maize line 1507, the control line, and a comparison of means. Any statistically significant differences ($\alpha = 0.05$) between the test line and the control line

were generally considered to have no nutritional effect, as long as the means for Cry1F maize line 1507 fell within the range published in the literature for commercial maize hybrids.

The forage samples were all dried to between 3 and 7 % moisture before processing. The grain was dried to between 11 and 13 % moisture before shelling. Woodson-Tenent determined the exact moisture content for each sample so the results could be reported on a dry weight basis.

1. Forage analysis

Proximate analysis of maize forage

An analysis of the protein, fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), carbohydrate, ash, and moisture levels of forage in hybrid maize line 1507 and the control hybrid was conducted and the results are shown in Table 13. The levels of protein, fat, carbohydrates, and ash in maize line 1507 were all within the reported literature ranges for maize forage. The published literature reports means for forage ADF and NDF are 30% and 51%, respectively; however, no range of values is available. The ADF and NDF levels in maize line 1507 of 26.91% and 48.01%, respectively, are similar to the means reported in the literature.

Conclusions

In summary, the analysis of nutrient composition of forage from maize line 1507 showed that it is comparable to forage from commercial maize hybrids.

2. Grain analysis

Proximate analysis of maize grain

An analysis of the protein, fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), carbohydrate, ash, and moisture levels of grain from hybrid maize line 1507 and the control hybrid was conducted and the results are shown in Table 14. The levels in maize line 1507 of protein, fat, ADF, NDF, carbohydrates, and ash were all within the literature range for maize grain.

Mineral analysis of maize grain

Levels of eight minerals (calcium, phosphorus, copper, iron, magnesium, manganese, potassium, and zinc) were analyzed in maize line 1507 and the control hybrid (Table 15). The mineral levels in maize line 1507 were within the literature range for maize grain.

Fatty acid composition of maize grain

Grain from hybrid maize line 1507 and the control hybrid was analyzed for the five major fatty acids in maize: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) (Table 16). The levels of the five fatty acids were all within the literature range for grain from maize line 1507.

Amino acid analysis of maize grain

Both the nutritionally essential and non-essential amino acids were analyzed in grain from maize line 1507 and the control hybrid (Table 17). With the exception of threonine, levels of all other essential amino acids were within the range published in the literature or determined by the analysis of 22 commercial Pioneer® Brand hybrids. Levels for threonine in both maize line 1507 (0.40 %) and the control hybrid (0.41 %) were slightly above the published range of 0.29 - 0.39 %. However, there was no statistically significant difference in threonine levels between the test and control lines, indicating that the slightly elevated level in maize line 1507 was not due to the presence of the transgenes. Levels for non-essential amino acids were within the expected range, except for glutamic acid. However, there was no statistically significant difference in glutamic levels between the test and control lines. A small in increase in levels of a non-essential amino acid would not have a detrimental impact on the nutritional quality of corn.

Vitamins analysis of maize grain

Grain from hybrid maize line 1507 and the control hybrid was analyzed for vitamin content (Table 18). Vitamin B1, vitamin B2, total tocopherols and folic acid levels were determined for comparison to the range of values found for maize in the literature, where available. Levels of vitamins B1, B2 and tocopherols were found to be within the published range for maize grain. There is no typical range available for folic acid in maize grain, although an

average value of 0.3 ppm is reported (Watson, 1987). Levels of folic acid in maize line 1507 and the control line were not significantly different across all locations ($p = 0.676$).

Secondary metabolites and anti-nutrients

Grain from hybrid maize line 1507 and the control hybrid was analyzed for secondary metabolites and two potential anti-nutrients (Table 19). There were no statistically significant differences between maize line 1507 and the control line for the levels of inositol, p-coumaric acid and ferulic acid. Levels of furfural were below the level of quantitation (0.500 mg/100 g) for both maize line 1507 and the control line. There were no literature ranges available for these four secondary metabolites. The levels of raffinose were within the range published in the literature and there were no significant differences between maize line 1507 and the control line.

Maize grain typically contains low concentrations of the anti- nutrient phytic acid (Cheryan, 1980). Phytic acid levels in maize line 1507 were found to be within the published literature range for maize grain. Maize usually contains only low levels of the anti-nutrient trypsin inhibitor (Watson, 1987; Del Valle, 1983). As expected, trypsin inhibitor levels in both maize line 1507 and the control line were below the limit of quantitation of the enzyme assay that was used in this analysis. This confirms that no unusually high levels of trypsin inhibitor are present in maize line 1507.

Conclusions

In summary, the analysis of nutrient composition of grain from maize line 1507 showed that it is comparable to grain from commercial maize hybrids.

V. REFERENCES

American Oil Chemists Society (AOCS) 1998. Official Methods and Recommended Practices of the AOCS. 5th edition. AOCS Press, Champaign, Illinois.

Association of Official Analytical Chemists (AOAC) 1990. Official Methods of Analysis. 15th edition. J. Helrich (ed.) AOAC, Arlington, Virginia.

Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye-Binding. *Anal. Biochem.* 72: 248-254.

Cheryan, M. 1980. Phytic acid and interactions in food systems. In *CRC Critical Review in Food Science and Nutrition.* pp. 297-335.

Del Valle, F.R., Pico, M.L., Camacho, J.L., Bourges, H. 1983. Effect of processing parameters on trypsin inhibitor and lectin contents of tortillas from whole raw corn-soybean mixtures. *J. Food Science* 48: 246-252.

FDA (Food and Drug Administration). 1992. U. S. Food and Drug Administration. Statement of policy: foods derived from new plant varieties. Federal Register (USA) 57:22984-23005.

IFBC. 1990. Safety Evaluation of Whole Foods and Other Complex Mixtures (Chapter 6). In: Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification. International Food Biotechnology Council. (eds. Coulston, F. and Kolbye, Jr., A.C.). Published in: Regulatory Toxicology and Pharmacology Volume 12, No. 3, December 1990. Academic Press, Inc.

Iowa Gold Catalog, 1994. Iowa Department of Agriculture and Land Stewardship. 1993 Catalog.

Iowa State University. 1993. How a Corn Plant Grows. ISU Cooperative Extension Service Special Publication 48.

Watson, S.A. 1982. Corn: Amazing Maize. General Properties. pp. 3-29. *In:* CRC Handbook of Processing and Utilization in Agriculture, Vol. II: Part 1 Plant Products. I.A. Wolff (ed.). CRC Press, Inc., Florida.

Watson, S.A. 1987. Structure and Composition. pp. 53-82. *In:* Corn: Chemistry and Technology, S.A. Watson and P.E. Ransted (eds.). American Association of Cereal Chemists, Inc., Minnesota.

Wych, R. D. Production of Hybrid Seed Corn. 1988. pg 603. In *Corn and Corn Improvement*, 3rd ed.; G.F. Sprage and J.W. Dudley, (eds). American Society of Agronomy, Inc. Wisconsin.

TABLE 1. FIELD PLOT MAP: BUIN, CHILE (BUI)

	Range 2[a]	Range 3
Row 5	(Block 1) Control Inbred A_M	(Block2) Control Hybrid A_M
Row 6	Control Inbred A_M	Control Hybrid A_M
Row 7	Control Inbred A_M	1507 Hybrid
Row 8	1507 Inbred	1507 Hybrid
Row 9	1507 Inbred	(Block3) 1507 Hybrid
Row 10	1507 Inbred	1507 Hybrid
Row 11	1362 Inbred	Control Hybrid A_M
Row 12	1362 Inbred	Control Hybrid A_M
Row 13	1362 Inbred	(Block4) 1507 Hybrid
Row 14	1362 Hybrid	1507 Hybrid
Row 15	1362 Hybrid	Control Hybrid A_M
Row 16	1362 Hybrid	Control Hybrid A_M
Row 17	Control Hybrid A_M	(Block5) Control Hybrid A_M
Row 18	Control Hybrid A_M	Control Hybrid A_M
Row 19	Control Hybrid A_M	1507 Hybrid
Row 20	1507 Hybrid	1507 Hybrid
Row 21	1507 Hybrid	(Block6) Control Hybrid A_M
Row 22	1507 Hybrid	Control Hybrid A_M
Row 23	filler	1507 Hybrid
Row 24	filler	1507 Hybrid

[a] Rows 5 to 13 of Range 2 contained inbred entries for the protein expression phase of this study. Rows 14 to 16 of Range 2 contained the 1362 hybrid. Data from these inbred and hybrid entries were not used in this study.

TABLE 2. FIELD PLOT MAP: VILUCO, CHILE (VIL)

	Range 2[a]	Range 3
Row 5	(Block 1) Control Inbred A_M	(Block2) Control Hybrid A_M
Row 6	Control Inbred A_M	Control Hybrid A_M
Row 7	Control Inbred A_M	1507 Hybrid
Row 8	1507 Inbred	1507 Hybrid
Row 9	1507 Inbred	(Block3) 1507 Hybrid
Row 10	1507 Inbred	1507 Hybrid
Row 11	1362 Inbred	Control Hybrid A_M
Row 12	1362 Inbred	Control Hybrid A_M
Row 13	1362 Inbred	(Block4) 1507 Hybrid
Row 14	1362 Hybrid	1507 Hybrid
Row 15	1362 Hybrid	Control Hybrid A_M
Row 16	1362 Hybrid	Control Hybrid A_M
Row 17	Control Hybrid A_M	(Block5) Control Hybrid A_M
Row 18	Control Hybrid A_M	Control Hybrid A_M
Row 19	Control Hybrid A_M	1507 Hybrid
Row 20	1507 Hybrid	1507 Hybrid
Row 21	1507 Hybrid	(Block6) Control Hybrid A_M
Row 22	1507 Hybrid	Control Hybrid A_M
Row 23	filler	1507 Hybrid
Row 24	filler	1507 Hybrid

[a] Rows 5 to 13 of Range 2 contained inbred entries for the protein expression phase of this study. Rows 14 to 16 of Range 2 contained the 1362 hybrid. Data from these inbred and hybrid entries were not used in this study.

TABLE 3. FIELD PLOT MAP: GRANEROS, CHILE (GRA)

	Range 2[a]	Range 3
Row 5	(Block 1) Control Inbred A_M	(Block2) Control Hybrid A_M
Row 6	Control Inbred A_M	Control Hybrid A_M
Row 7	Control Inbred A_M	1507 Hybrid
Row 8	1507 Inbred	1507 Hybrid
Row 9	1507 Inbred	(Block3) 1507 Hybrid
Row 10	1507 Inbred	1507 Hybrid
Row 11	1362 Inbred	Control Hybrid A_M
Row 12	1362 Inbred	Control Hybrid A_M
Row 13	1362 Inbred	(Block4) 1507 Hybrid
Row 14	1362 Hybrid	1507 Hybrid
Row 15	1362 Hybrid	Control Hybrid A_M
Row 16	1362 Hybrid	Control Hybrid A_M
Row 17	Control Hybrid A_M	(Block5) Control Hybrid A_M
Row 18	Control Hybrid A_M	Control Hybrid A_M
Row 19	Control Hybrid A_M	1507 Hybrid
Row 20	1507 Hybrid	1507 Hybrid
Row 21	1507 Hybrid	(Block6) Control Hybrid A_M
Row 22	1507 Hybrid	Control Hybrid A_M
Row 23	filler	1507 Hybrid
Row 24	filler	1507 Hybrid

[a] Rows 5 to 13 of Range 2 contained inbred entries for the protein expression phase of this study. Rows 14 to 16 of Range 2 contained the 1362 hybrid. Data from these inbred and hybrid entries were not used in this study.

TABLE 4. FIELD PLOT MAP: NANCAGUA, CHILE (NAN)

	Range 2[a]	Range 3
Row 5	(Block 1) Control Inbred A_M	(Block2) Control Hybrid A_M
Row 6	Control Inbred A_M	Control Hybrid A_M
Row 7	Control Inbred A_M	1507 Hybrid
Row 8	1507 Inbred	1507 Hybrid
Row 9	1507 Inbred	(Block3) 1507 Hybrid
Row 10	1507 Inbred	1507 Hybrid
Row 11	1362 Inbred	Control Hybrid A_M
Row 12	1362 Inbred	Control Hybrid A_M
Row 13	1362 Inbred	(Block4) 1507 Hybrid
Row 14	1362 Hybrid	1507 Hybrid
Row 15	1362 Hybrid	Control Hybrid A_M
Row 16	1362 Hybrid	Control Hybrid A_M
Row 17	Control Hybrid A_M	(Block5) Control Hybrid A_M
Row 18	Control Hybrid A_M	Control Hybrid A_M
Row 19	Control Hybrid A_M	1507 Hybrid
Row 20	1507 Hybrid	1507 Hybrid
Row 21	1507 Hybrid	(Block6) Control Hybrid A_M
Row 22	1507 Hybrid	Control Hybrid A_M
Row 23	filler	1507 Hybrid
Row 24	filler	1507 Hybrid

[a] Rows 5 to 13 of Range 2 contained inbred entries for the protein expression phase of this study. Rows 14 to 16 of Range 2 contained the 1362 hybrid. Data from these inbred and hybrid entries were not used in this study.

TABLE 5. IMPORTANT CROP DATES FOR THE 1998/99 FIELD TRIALS CONDUCTED IN CHILE

Field Site Site Code	Seed Receipt	Planting	Pollinations	Harvest
Buin, Chile BUI	prior to 10/28/98	11/4/98	1/19/99 – 2/3/99	4/23/99
Viluco, Chile VIL	prior to 10/28/98	10/30/98	1/11/99 – 1/21/99	4/26/99
Graneros, Chile GRA	prior to 10/28/98	12/2/98	2/3/99 – 2/11/99	5/12/99
Nancagua, Chile NAN	prior to 10/28/98	12/2/98	2/3/99 – 2/10/99	5/12/99

TABLE 6. CHEMICAL AND FERTILIZER APPLICATIONS FOR THE 1998/99 FIELD TRIALS CONDUCTED IN BUIN, CHILE

Date	Product	Rate (kg ai/ha)
11/3/98	cyanazine	1.5
11/3/98	chlorpyrifos	2.9
11/3/98	metolachlor	1.9
11/4/98	N-P-K	112-95-81
11/4/98	carbofuran	1.0
11/4/98	flutriafol + carbofuran	0.05 + 0.60
11/20, 26/98	chlorpyrifos + cypermethrin	0.5 + 0.05
12/14/98	N-P-K	180-0-0
1/9, 20/99	lambda-cyhalothrin	0.01

TABLE 7. CHEMICAL AND FERTILIZER APPLICATIONS FOR THE 1998/99 FIELD TRIALS CONDUCTED IN VILUCO, CHILE

Date	Product	Rate (kg ai/ha)
10/29/98	cyanazine	1.5
10/29/98	chlorpyrifos	2.9
10/29/98	metolachlor	1.9
10/30/98	N-P-K	112-95-81
10/30/98	carbofuran	1.0
10/30/98	flutriafol + carbofuran	0.05 + 0.60
11/12,18, 25/98	chlorpyrifos + cypermethrin	0.5 + 0.05
11/19/98	bentazon	1.4
12/7/98	N-P-K	184-0-0
1/5, 13, 21, 29/99	lambda-cyhalothrin	0.01
2/23/99	dicofol	0.9
3/1/99	dicofol	0.7

TABLE 8. CHEMICAL AND FERTILIZER APPLICATIONS FOR THE 1998/99 FIELD TRIALS CONDUCTED IN GRANEROS, CHILE

Date	Product	Rate (kg ai/ha)
12/2/98	chlorpyrifos	3.4
12/2/98	atrazine	1.5
12/2/98	acetochlor	1.9
12/2/98	N-P-K	91-55-36
12/12, 19, 30/98	chlorpyrifos	1.4
12/27/98	N-P-K	138-0-0
2/26/99	dicofol	1.0
2/26/99	lambda-cyhalothrin	0.01

TABLE 9. CHEMICAL AND FERTILIZER APPLICATIONS FOR THE 1998/99 FIELD TRIALS CONDUCTED IN NANCAGUA, CHILE

Date	Product	Rate (kg ai/ha)
12/1/98	chlorpyrifos	2.8
12/1/98	acetochlor	2.3
12/1/98	atrazine	1.7
12/2/98	chlorpyrifos	1
12/2/98	N-P-K	91-55-36
12/12, 18, 30/98	chlorpyrifos	1.4
12/31/98	N-P-K	138-0-0
2/27/99	dicofol	0.7
2/27/99	lambda-cyhalothrin	0.01

TABLE 10. SUMMARY OF GLUFOSINATE-AMMONIUM HERBICIDE SEGREGATION

		Buin		Viluco		Granero		Nancagua	
	entry	plants emerged	surviving plants	plants emerged	surviving plants	plants emerged	surviving plants	plants emerged	surviving plants
BLOCK 1	1507I	54-57	29	54-57	21	53	25	54	29
	1507H	54-57	35	54-57	37	49	35	53	37
Block 2	1507H	36-38	20	36-38	19	39	23	39	21
Block 3	1507H	38-38	18	36-38	20	40	23	8	22
Block 4	1507H	36-38	22	36-38	21	38	21	38	19
Block 5	1507H	36-38	22	36-38	20	36	23	36	20
Block 6	1507H	36-38	19	36-38	22	42	21	43	27

TABLE 11. SUMMARY OF RAINFALL AND TEMPERATURES FOR 1998/99 GROWING SEASON.

Month	Parameter	BUI	VIL	GRA	NAN
November	Maximum temperature - °F	88	92	not available	not available
	Minimum temperature - °F	39	39		
	Average temperature - °F	62	64		
	Total Rainfall – inches[1]	0.0	0.0		
December	Maximum temperature - °F	97	97	93	99
	Minimum temperature - °F	45	45	44	44
	Average temperature - °F	68	69	68	71
	Total Rainfall – inches[1]	0.0	0.0	not available	not available
January	Maximum temperature - °F	94	93	91	100
	Minimum temperature - °F	45	45	43	44
	Average temperature - °F	67	67	68	71
	Total Rainfall – inches[1]	0.0	0.0	not available	not available
February	Maximum temperature - °F	93	93	93	97
	Minimum temperature - °F	44	45	42	44
	Average temperature - °F	68	68	68	71
	Total Rainfall – inches[1]	0.0	0.0	not available	not available
March	Maximum temperature - °F	88	87	90	91
	Minimum temperature - °F	40	42	37	38
	Average temperature - °F	63	64	64	68
	Total Rainfall – inches[1]	5.8	5.8	not available	not available
April	Maximum temperature - °F	83	83	not available	not available
	Minimum temperature - °F	32	35		
	Average temperature - °F	58	58		
	Total Rainfall – inches[1]	0.06	0.06		

1 Natural precipitation was supplemented by in-furrow irrigation as required to produce a normal crop. However, the amount of irrigation applied was not recorded.

TABLE 12. SUMMARY OF TISSUE SAMPLING DATES FOR THE 1998/1999 FIELD TRIALS

Field Site Site Code	Whole Plant	Grain
Buin, Chile BUI	3/11, 12/99	4/23/99
Viluco, Chile VIL	3/1, 2/99	4/26/99
Graneros, Chile GRA	4/6/99	5/12/99
Nancagua, Chile NAN	3/31/99	5/12/99

TABLE 13. PROXIMATE ANALYSIS – FORAGE

The means and p-values for the proximate analysis of forage from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Proximate Analysis of Forage			
Response Variable[a]	**Treatment Estimates (Mean)**	**Treatment Effect p-value**	**Range of Values in Literature[b]**
Fat %	Line 1507 mean = 2.42 Control line mean = 2.42	0.997	0.7 - 6.7
Protein %	Line 1507 mean = 7.82 Control line mean = 7.81	0.958	3.5 - 15.9
ADF %	Line 1507 mean = 26.91 Control line mean = 27.56	0.097	30[c]
NDF %	Line 1507 mean = 48.01 Control line mean = 48.21	0.766	51[c]
Carbohydrates %	Line 1507 mean = 84.15 Control line mean = 84.13	0.9.25	66.9 – 94.5[d]
Ash %	Line 1507 mean = 5.61 Control line mean = 5.63	0.848	1.3 - 10.5

a – Percentage on a dry weight basis
b – Watson, 1982
c – Watson, 1982 reports an average value for ADF of 30% and NDF of 51%.
d – Carbohydrates are calculated as the percentage of dry weight =100% - % protein - % fat - % ash. Fiber (ADF and NDF) is included in the carbohydrates.

TABLE 14. PROXIMATE ANALYSIS – GRAIN

The means and p-values for the proximate analysis of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Proximate Analysis of Grain			
Response Variable[a]	**Treatment Estimates (Mean)**	**Treatment Effect p-value**	**Range of Values in Literature**
Fat %	Line 1507 mean = 3.83 Control line mean = 3.94	0.046	3.1 - 5.7[b]
Protein %	Line 1507 mean = 11.20 Control line mean = 11.32	0.611	6.0 – 12[b]
ADF %	Line 1507 mean = 3.55 Control line mean = 3.68	0.250	3.0 – 4.3[c]
NDF %	Line 1507 mean = 10.47 Control line mean = 10.08	0.315	8.3 – 11.9[b]
Carbohydrates %[d]	Line 1507 mean = 83.45 Control line mean = 83.23	0.352	63.3 – 89.7[c]
Ash %	Line 1507 mean = 1.51 Control line mean = 1.50	0.335	1.1 - 3.9[c]

a – Percentage on a dry weight basis.
b _ Watson, 1987.
c _ Watson, 1982.
d – Carbohydrates are calculated as the percentage of dry weight =100% - % protein - % fat - % ash. Fiber (ADF and NDF) is included in the carbohydrates.

TABLE 15. MINERAL COMPOSITION – GRAIN

The means and p-values for the mineral composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Mineral Analyses of Grain			
Response Variable[a]	**Treatment Estimates**	**Treatment Effect p-value**	**Range of Values in Literature[c]**
Calcium %	Line 1507 mean = 0.0036 Control line mean = 0.0031	0.620	0.01 - 0.10 0.002 - 0.011[d]
Phosphorous %	Line 1507 mean = 0.33 Control line mean = 0.32	0.161	0.26 - 0.75
Copper ppm	Line 1507 mean = 2.03 Control line mean = 2.11	0.845	0.9 – 10
Iron %	Line 1507 mean = 0.0025 Control line mean = 0.0025	0.549	0.0001 – 0.01
Magnesium %	Line 1507 mean = 0.12 Control line mean = 0.13	0.524	0.09 - 1.0
Manganese %	Line 1507 mean = 0.0005 Control line mean = 0.0006	0.0003	0.00007 – 0.0054
Potassium %	Line 1507 mean = 0.40 Control line mean = 0.36	0.023	0.32 – 0.72
Sodium %	Line 1507 mean = < LOQ[b] Control line mean = < LOQ	–	0.0 – 0.15
Zinc %	Line 1507 mean = 0.002 Control line mean = 0.002	0.141	0.0012 – 0.0030

a _ Percentage on a dry weight basis or parts per million (ppm) on a dry weight basis.
b – Below the level of quantitation for sodium of 0.01%
c - Watson, 1982.
d – Data from analysis of 22 commercial Pioneer® Brand Hybrids.

TABLE 16. FATTY ACID COMPOSITION – GRAIN

The means and p-values for the fatty acid composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Fatty Acid Analyses of Grain			
Response Variable[a]	**Treatment Estimates**	**Treatment Effect p-value**	**Range of Values in Literature[b]**
Palmitic %	Line 1507 mean = 11.07 Control line mean = 10.92	0.091	7 - 19
Stearic %	Line 1507 mean = 2.28 Control line mean = 2.44	0.007	1 - 3
Oleic %	Line 1507 mean = 30.61 Control line mean = 32.53	0.002	20 - 46
Linoleic %	Line 1507 mean = 53.10 Control line mean = 51.16	0.002	35 - 70
Linolenic %	Line 1507 mean = 1.29 Control line mean = 1.21	0.0001	0.8 - 2

a – Value of fatty acid is % of total lipid.
b – Watson, 1982.
c - "Within range" refers to whether the treatment estimate for maize line 1507 is within the range given for maize.

TABLE 17. AMINO ACID COMPOSITION – GRAIN

The means and p-values for the amino acid composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Amino Acid Analyses of Grain			
Essential Amino Acids			
Response Variable*	**Treatment Estimates**	**Treatment Effect p-value**	**Range of Values in Literature**
Glycine	Line 1507 mean = 0.39 Control line mean = 0.40	0.150	0.26 - 0.47[b] 0.24 - 0.41[c]
Threonine	Line 1507 mean = 0.40 Control line mean = 0.41	0.302	0.29 - 0.39[b] 0.21 - 0.37[c]
Valine	Line 1507 mean = 0.51 Control line mean = 0.52	0.902	0.21 - 0.52[b] 0.25 - 0.67[c]
Isoleucine	Line 1507 mean = 0.40 Control line mean = 0.40	0.952	0.26 - 0.40[b] 0.19 - 0.39[c]
Leucine	Line 1507 mean = 1.42 Control line mean = 1.43	0.880	0.78 -1.52[b] 0.43 - 1.35[c]
Phenylalanine	Line 1507 mean = 0.56 Control line mean = 0.57	0.479	0.29 - 0.57[b] 0.04 - 0.54[c]
Histidine	Line 1507 mean = 0.29 Control line mean = 0.30	0.822	0.20 - 0.28[b] 0.21 - 0.32[c]
Lysine	Line 1507 mean = 0.32 Control line mean = 0.32	0.522	0.20 - 0.38[b] 0.19 - 0.36[c]
Arginine	Line 1507 mean = 0.44 Control line mean = 0.45	0.672	0.29 - 0.59[b] 0.28 - 0.55[c]

Table 17. Amino acid composition - Grain (continued)

Response Variable[a]	Treatment Estimates	Treatment Effect p-value	Range of Values in Literature
Cysteine	Line 1507 mean = 0.21 Control line mean = 0.23	<0.0001	0.12 - 0.16[b] 0.13 - 0.27[c]
Methionine	Line 1507 mean = 0.19 Control line mean = 0.20	0.020	0.10 - 0.21[b] 0.12 - 0.26[c]
Tryptophan	Line 1507 mean = 0.08 Control line mean = 0.08	0.065	0.05 - 0.12[b] 0.05 - 0.10[c]
Non-essential Amino Acids			
Serine	Line 1507 mean = 0.54 Control line mean = 0.55	0.390	0.42 - 0.55[b] 0.25 - 0.46[c]
Alanine	Line 1507 mean = 0.84 Control line mean = 0.85	0.727	0.64 - 0.99[b] 0.37 - 0.81[c]
Glutamic Acid	Line 1507 mean = 2.14 Control line mean = 2.18	0.472	1.24 - 1.96[b] 0.89 - 2.02[c]
Proline	Line 1507 mean = 1.01 Control line mean = 1.03	0.679	0.66 - 1.03[b] 0.43 - 1.01[c]
Aspartic Acid	Line 1507 mean = 0.77 Control line mean = 0.81	0.102	0.58 - 0.72[b] 0.37 - 0.80[c]
Tyrosine	Line 1507 mean = 0.20 Control line mean = 0.20	0.954	0.29 - 0.47[b] 0.17 - 0.31[d]

a – Percentage on a dry weight basis.
b - Watson, 1982.
c – Data from analysis of 22 commercial Pioneer® Brand Hybrids.
d - Iowa Gold Catalog, 1994.

TABLE 18. VITAMIN COMPOSITION – GRAIN

The means and p-values for the vitamin composition of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Vitamin Analyses of Grain			
Response Variable[a]	**Treatment Estimates**	**Treatment Effect p-value**	**Range of Values in Maize[b]**
Thiamine Hydrochloride (B1) ppm	Line 1507 mean = 3.64 Control line mean = 4.06	0.002	3.0 – 8.6
Riboflavin (B2) ppm	Line 1507 mean = 1.67 Control line mean = 1.66	0.827	0.25 – 5.6
Folic Acid ppm	Line 1507 mean = 0.151 Control line mean = 0.144	0.676	0.3[c]
Total tocopherols ppm	Line 1507 mean = 48.2 Control line mean = 41.6	0.0005	42 - 87

a – Parts per million (ppm) on a dry weight basis.
b – Watson, 1982
c – Watson, 1987 reports an average value for folic acid in grain as 0.3 mg/kg.

TABLE 19. SECONDARY METABOLITES AND ANTI-NUTRIENTS– GRAIN.

The means and p-values for the secondary metabolite and anti-nutrients of grain from maize line 1507 and the control hybrid for samples collected in the 1998/1999 field trial. The range of values found in the literature for each trait is listed.

Secondary Metabolites in Grain			
Response Variable[a]	**Treatment Estimates**	**Treatment Effect p-value**	**Range of Values in Maize[b]**
Inositol mg/100 g	Line 1507 mean = 59.96 Control line mean = 61.68	0.681	NA
Raffinose %	Line 1507 mean = 0.086 Control line mean = 0.087	0.685	0.08 – 0.30
P-Coumaric Acid mg/100 g	Line 1507 mean = 16.5 Control line mean = 16.0	0.547	NA
Furfural mg/100g	Line 1507 mean = <LOQ[c] Control line mean = <LOQ	--	NA
Ferulic Acid mg/100 g	Line 1507 mean = 264 Control line mean = 257	0.612	NA
Anti-nutrient Analyses of Grain			
Phytic acid %	Line 1507 mean = 0.99 Control line mean = 0.96	0.146	0.7 – 1.0
Trypsin Inhibitor TIU/g	Line 1507 mean = < LOQ[d] Control line mean = < LOQ	--	NA

a – Percentage (%) or mg/100 g on a dry weight basis. Trypsin inhibitor is expressed in units of trypsin inhibitor enzyme activity per gram on a dry basis.
b _ Watson, 1982.
c – Below level of quantitation for furfural of 0.500 mg/100 g.
d _ Below level of quantitation for trypsin inhibitor of 2000 TIU/g.
NA _ Ranges are not available in the published literature.