



Request for Extension of Determination of Nonregulated Status to the Additional Regulated Article:

Maize Line HCEM485

The undersigned submits this request under 7 CFR Part 340.6(e) to request an extension of determination of nonregulated status that the article should not be regulated under 7 CFR Part 340.

Submitted by:

(b)(6)

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**Submitted:
2 March 2009**

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Stine Petition #SSF-09-061

Summary

Glyphosate herbicide-tolerant maize line HCEM485 was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene. DNA introduction was via aerosol beam injector, which is a naked DNA delivery method.

The 6.0 kb fragment contained the endogenous maize *epsps* expression cassette including native promoter, coding sequence, intron, and termination regions. The maize EPSPS coding sequence was specifically modified by site-directed mutagenesis to introduce two single-nucleotide substitutions. These two point mutations resulted in a codon change from threonine→isoleucine at position 102 (relative to the amino acid sequence of the native maize EPSPS enzyme) and a proline→serine change at position 106. These two amino acid substitutions result in a glyphosate-tolerant form of the enzyme and are also present in the modified EPSPS enzyme produced in the antecedent organism, transgenic maize event GA21. Except for the amino acid substitutions at positions 102 and 106, the amino acid sequence of the double-mutated EPSPS (2mEPSPS) enzyme is identical to the native maize EPSPS sequence.

The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-tolerant form of the native EPSPS enzyme. Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species, including those that could be considered a plant pest. In addition, the genetic modification process resulting in maize line HCEM485 did not employ any organism (*e.g.*, *Agrobacterium tumefaciens*) that could be considered a plant pest.

The introduced sequences in maize line HCEM485 are contained within a single genetic locus within the maize genome as demonstrated by Southern blot analysis and Mendelian inheritance studies. The modified maize EPSP synthase expressed in maize line HCEM485 is intact, of the expected molecular weight and there was no evidence of truncated forms of the enzyme. The modified maize EPSPS expressed in HCEM485 maize is also immunochemically cross-reactive with the modified maize EPSPS expressed in the antecedent organism, GA21, and the enzymes from both sources express the same mutations responsible for conferring glyphosate herbicide tolerance.

Agronomic and phenotypic characteristics of an HCEM485 maize hybrid and three control hybrids were evaluated in a series of field trials across 15 United States Corn Belt locations in 2007. The agronomic characteristics chosen for comparison were those typically observed by professional maize breeders and agronomists and represented a broad range of characteristics throughout the development of the maize plant. Results of these trials suggest that there were no biologically significant unintended effects on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight, or disease susceptibility as a result of the genetic modification introduced into maize line HCEM485. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of outcrossing than unmodified maize.

Levels of key nutrients, minerals, antinutrients, and secondary metabolites were determined in samples of maize grain and forage derived from HCEM485 and control hybrids collected from up to four field trial locations in 2007. For most analyses, there were no statistically significant differences and in cases where statistically significant differences were observed, the magnitudes of the differences were small and in every case, mean values determined for both HCEM485 and control samples were within the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically significant changes in composition of the grain or forage had occurred as an unintended consequence of the genetic modification resulting in maize line HCEM485. The conclusion based on these data was that grain and forage from HCEM485 maize were substantially equivalent in composition to both the control hybrids included in this study, and to other commercial maize hybrids.

In conclusion, there is no expectation that cultivation of maize line HCEM485 would have any environmental effects different from the cultivation of the antecedent organism, GA21, or other maize lines exhibiting glyphosate tolerance that have also been deregulated by USDA-APHIS (*e.g.*, NK603; MON 88017; and MON 802). Therefore, on the basis of the substantial phenotypic equivalence between maize line HCEM485 and the antecedent organism, GA21, Stine Seed Farm requests that an extension of nonregulated status be granted to maize line HCEM485, any progeny derived from crosses between HCEM485 and conventional maize, and any progeny derived from crosses of HCEM485 with other deregulated maize lines.

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Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner which are unfavorable to the petition.

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Abbreviations Used in This Petition

2mEPSPS	double-mutated EPSP synthase; native maize EPSPS containing Thr-102→Ile and Pro-106→Ser substitutions.
AI	active ingredient
APHIS	Animal and Plant Health Inspection Service
BAC	bacterial artificial chromosome
bp	base pairs
CBI	confidential business information
CTP	chloroplast transit peptide
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	Food and Drug Administration
FW	fresh weight
g	gram
GRAS	generally recognized as safe
HRP	horseradish peroxidase
ILSI	International Life Sciences Institute
kb	kilobases
kg	kilogram
LOQ	limit of quantification
µg	microgram
mg	milligram
µm	micrometer
MW	molecular weight
ND	not determined
NOS	nopaline synthase
OECD	Organization for Economic Cooperation and Development
OTP	optimized transit peptide
PEP	phosphoenolpyruvate
S3P	shikimate-3-phosphate
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TIPS	threonine to isoleucine mutation at position 102 and proline to serine mutation at position 106, relative to the amino acid sequence of the native maize EPSP synthase enzyme.
USDA	United States Department of Agriculture

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Annexes

Note: The following annexes are included as attachments.

Annex 1

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Annex 2

Annex 3

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I. RATIONALE FOR SUBMISSION OF REQUEST FOR EXTENSION OF NONREGULATED STATUS

I.1 BASIS FOR THE REQUEST

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has been given the responsibility, under the Federal Plant Pest Act (7 U.S.C. 150aa–150jj) and the Plant Quarantine Act (7 U.S.C. 151–167), to prevent the introduction and dissemination into the United States or interstate of plant pests. Under this authority, APHIS has published regulations found at 7 CFR Part 340 pertaining to the introduction (importation, interstate movement, and release into the environment) of genetically engineered organisms and products derived from known plant pests (regulated articles). An organism is not subject to the regulations when the organism is demonstrated not to present a plant pest risk.

Section 340.6(e) of the regulations provides that APHIS may extend a determination of non-regulated status to additional articles, upon finding that the additional articles do not pose a potential for plant pest risk, and should therefore not be regulated. Such a finding would be made based on an evaluation of the similarity of the additional articles to an antecedent organism, *i.e.*, an organism that has already been the subject of a determination of nonregulated status by APHIS under Section 340.6, and that is used as a reference for comparison to the subject article under consideration.

In its guidance, APHIS has provided the following example of a molecular manipulation that is unlikely to pose new risk issues beyond those that would have been considered in the initial determination of nonregulated status:

- *Modifications in which the amino acid sequence of any encoded proteins is unchanged with respect to the corresponding sequence in the antecedent organism (i.e., synonymous codon changes).*

When applying this guidance it is clear that a request for an extension of determination of nonregulated status for maize line HCEM485 as based upon the previous determination of nonregulated status for Roundup Ready® maize line GA21 (petition 97-099-01p) is appropriate. The glyphosate tolerance of maize line GA21 was imparted by the insertion of a double-mutated form of the maize (*Zea mays* L.) EPSPS encoding gene into the maize genome. In the same manner, the glyphosate tolerance in maize line HCEM485 is also based on expression of the same modified EPSPS enzyme derived from *Z. mays*, with the notable difference that expression of the modified EPSPS enzyme is regulated by endogenous DNA sequences also derived from *Z. mays* rather than regulatory sequences derived from other species.

The specific differences between HCEM485 and its progeny, and the event GA21 in the previous petition are discussed in the appropriate sections and also summarized in Table 1.

As a further basis for this request for a determination of nonregulated status, the petitioner notes that glyphosate tolerant maize line HCEM485 contains only DNA sequences derived from the recipient organism, *Z. mays*, which is not considered a plant pest; it does not contain DNA sequences derived from any organism that could be considered to pose a plant pest risk nor was it produced using any organism that could be considered to pose a plant pest risk. On

this basis, it can be concluded that maize line HCEM485 is unlikely to pose a plant pest risk and may, therefore, be afforded nonregulated status.

Table 1: Comparison of maize line HCEM485 with event GA21

Characteristic	HCEM485	Event GA21
Crop	maize	maize
Genus and species name	<i>Zea mays</i>	<i>Zea mays</i>
Parent line	Stine 963	Unspecified
Transformation method	Aerosol beam direct DNA transfer	Microparticle acceleration direct DNA transfer
Trait	Tolerance to glyphosate herbicide	Tolerance to glyphosate herbicide
Gene product	double-mutated EPSPS (2mEPSPS)	double-mutated EPSPS
Vector	pHCEM	pDPG434
Transforming DNA	<i>Cla</i> I + <i>Eco</i> RV restriction fragment (ca. 6.0 kb)	<i>Not</i> I restriction fragment (ca. 3.4 kb)
Gene and source	Modified EPSPS-encoding gene from <i>Z. mays</i> including native introns and exons	Modified EPSPS-encoding gene from <i>Z. mays</i>
Targeting sequences	Native chloroplast transit sequences from <i>Z. mays</i> EPSPS-encoding gene	Optimized chloroplast transit sequences derived from <i>Z. mays</i> and <i>Helianthus annuus</i> (sunflower) ribulose-1,5-bisphosphate carboxylase genes
Promoter and source	5' region of the maize (<i>Z. mays</i>) EPSPS-encoding gene containing native promoter sequences	5' region of the rice (<i>Oryza sativa</i>) actin 1 gene containing the promoter and first intron
Terminator and source	3' nontranslated region of the native maize (<i>Z. mays</i>) EPSPS-encoding gene	3' nontranslated region from the nopaline synthase (<i>nos</i>) gene derived from the Ti plasmid of <i>Agrobacterium tumefaciens</i>

I.2 RATIONALE FOR THE DEVELOPMENT OF MAIZE LINE HCEM485

There are no changes in rationale from Section I.A of the previously approved petition number 97-099-01-p, which briefly discusses the benefits of glyphosate tolerant maize.

Prior to commercialization of maize line HCEM485, Stine Seed Farm will seek the following regulatory approvals in the United States:

1. Extension of the existing determination of nonregulated status granted for maize line GA21 (97-099-01p) to maize line HCEM485 and all progenies from crosses between this line and other maize varieties.
2. Maize line HCEM485 is within the scope of the FDA policy statement concerning products derived from new plant varieties, including those genetically engineered, published in the Federal Register on May 29, 1992.

II. THE MAIZE FAMILY

There are no changes from Section II of the previously approved petition number 97-099-01p.

III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

The antecedent organism, maize line GA21, was produced using a particle acceleration method. For the production of maize line HCEM485, DNA introduction was via aerosol beam injector (Held *et al.*, 2004), which is a naked DNA delivery method.

III.1 DONOR GENES AND REGULATORY SEQUENCES

The antecedent organism, maize line GA21, was generated using a particle acceleration transformation system with a gel-isolated *NotI* DNA restriction fragment of plasmid vector pDPG434 containing the modified EPSPS encoding gene. In comparison, glyphosate-tolerant maize line HCEM485 was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene (Held *et al.*, 2006).

The maize BAC library was screened with a DNA probe complementary to a portion of the maize EPSP synthase gene (GenBank Accession No. X63374) and one of the resultant BAC clones containing a 6.0 kb genomic fragment flanked by unique *ClaI* and *EcoRV* restriction endonuclease sites was chosen for further characterization (Figure 1). Nucleotide sequencing of the 6.0 kb fragment revealed that it contained an *epsps* 5' regulatory sequence (before position 1868), an EPSP synthase coding region (positions 1868–5146) comprised of 8 exons (labelled a–h in Figure 1) and 7 introns, and a 3' untranslated region (after position 5146). The EPSP synthase coding region also contained sequences encoding an endogenous N-terminal chloroplast transit peptide (position 1868–2041) as predicted using the PSORT algorithm (Human Genome Center, Institute for Medical Science, University of Tokyo).

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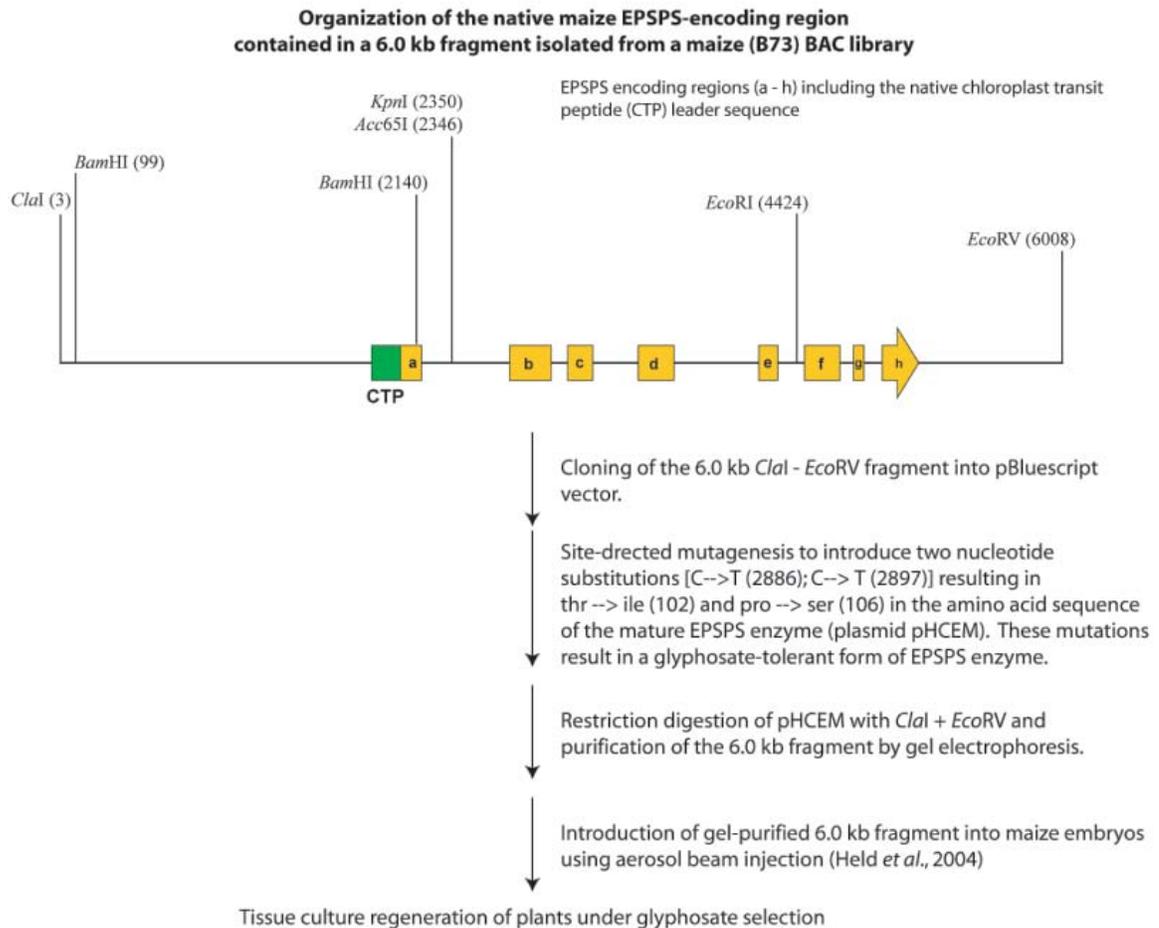


Figure 1: Map of 6.0 kb maize genomic fragment and developmental steps in creating maize line HCEM485.

III.2 THE VECTOR pHCEM

The 6.0 kb maize genomic fragment was cloned into the *Clal* and *EcoRV* sites of pBlueScript vector and subjected to site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). Two mutations were introduced into the EPSPS coding sequence: a cytosine to thymine substitution at position 2886 and a second cytosine to thymine substitution at position 2897. These two point-mutations resulted in two amino acid changes within the sequence of the mature EPSPS protein, a Thr-102→Ile and Pro-106→Ser substitution. The introduction of the T102I/P106S (TIPS) mutations was based on previous work demonstrating that Class I EPSP synthase variants containing TIPS mutations resulted in functional tolerance to glyphosate-containing herbicides (Spencer *et al.*, 2000; Lebrun *et al.*, 2003). These two mutations are the same mutations as introduced into the modified maize EPSP synthase encoding gene introduced into the antecedent organism, maize line GA21. An amino acid alignment of maize EPSP synthase enzymes illustrating these changes is included in Figure 2.

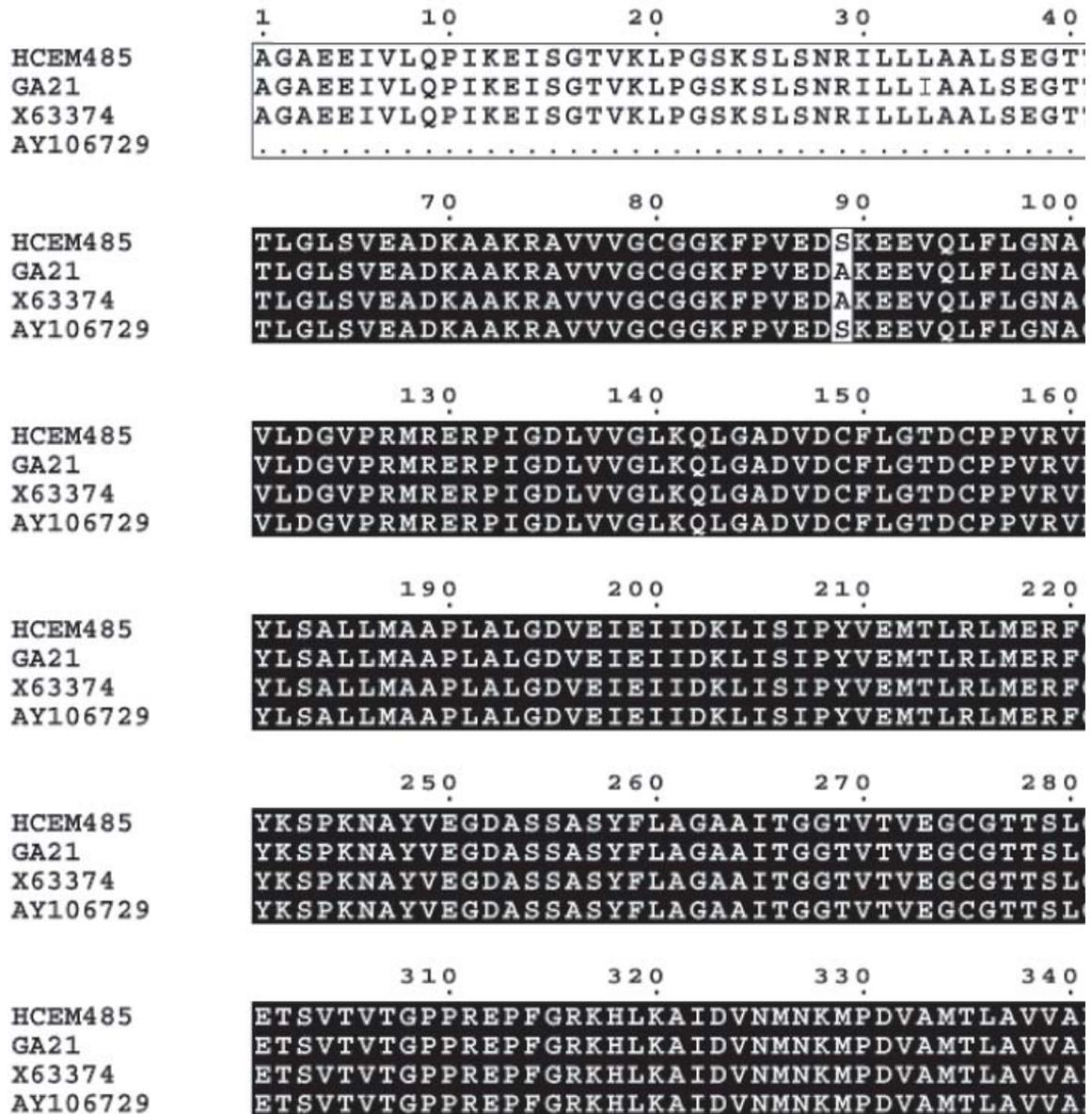


Figure 2: Amino acid sequence alignments of maize EPSP synthase enzymes. HCEM485 is the amino acid sequence of the mutated EPSP synthase expressed in maize line HCEM485 (Held *et al.*, 2006). GA21 is the amino acid sequence of the mutated EPSP synthase expressed in maize event GA21 (Spencer *et al.*, 2000). GenBank Accession No. X63374 corresponds to an EPSP synthase encoding sequence from a maize cell culture (Lebrun *et al.*, 1991). GenBank Accession No. AY106729 was identified from a maize bacterial artificial chromosome (BAC) library as part of a project of expressed sequence tag (EST) assemblies (Gardiner *et al.*, 2004). Positions of the threonine to isoleucine and proline to serine substitutions at positions 102 and 106 (relative to the native enzyme), respectively, are shown. The serine residue at position 89 of the HCEM485 EPSP synthase sequence is identical to the sequence of the native enzyme from GenBank Accession No. AY106729.

The pBlueScript vector containing the 6.0 kb maize genomic fragment with double-mutated EPSPS-encoding gene is designated pHCEM (Figure 3). Nucleotide sequencing of the mutated maize 6.0 kb fragment contained in pHCEM confirmed that no other alterations in sequence had been inadvertently introduced. This sequence is presented in Appendix 1.

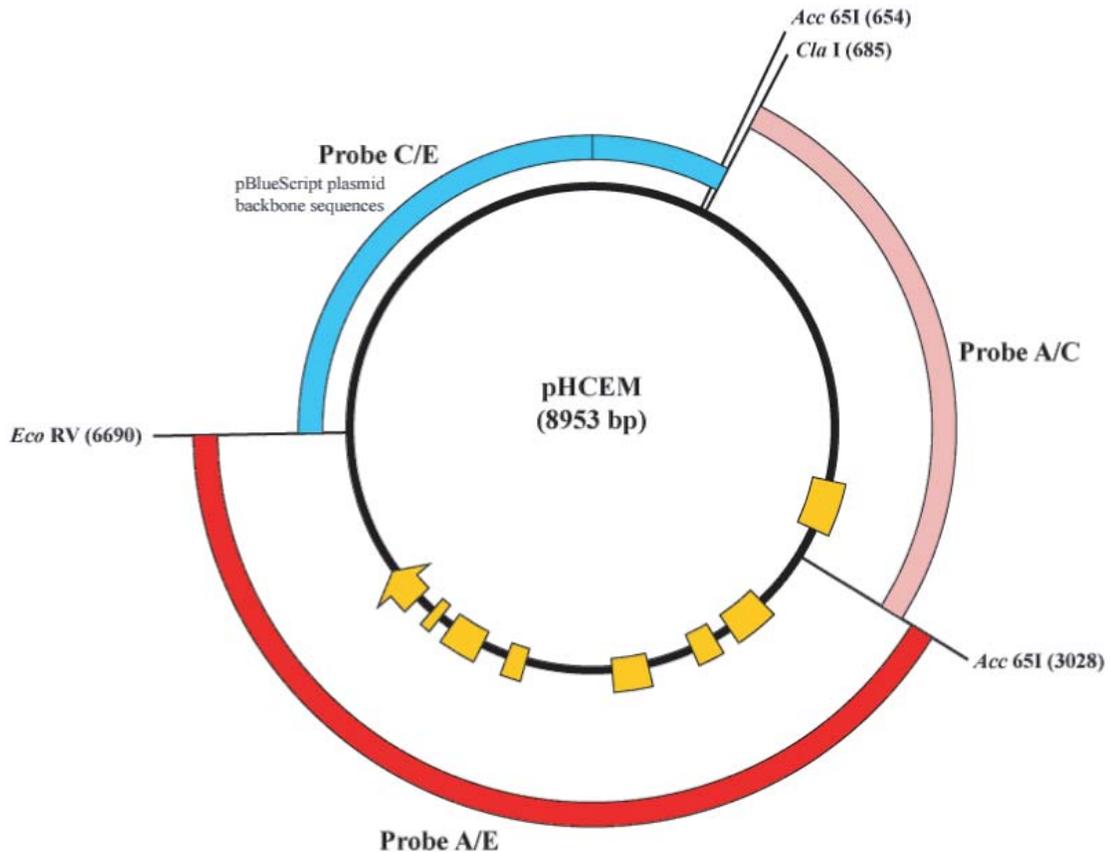


Figure 3: Plasmid map of pHCEM. Map of plasmid pHCEM containing the 6.0 kb *ClaI* – *EcoRV* fragment cloned into pBlueScript vector. The positions of relevant restriction endonuclease sites and of probes used in Southern hybridization analyses are indicated with numbering relative to the plasmid DNA sequence.

III.3 PLANT TRANSFORMATION AND REGENERATION

For DNA introduction, pHCEM was digested with *ClaI* and *EcoRV*, subjected to agarose gel electrophoresis (1 percent agarose), and the 6.0 kb band was excised and purified using Qia-gen's Qiaquick gel extraction kit. The purified maize DNA fragment was introduced into immature maize embryos derived from the elite inbred line Stine 963 by aerosol beam injection (Held *et al.*, 2004). After 5 days of culture on non-selective medium, embryos were transferred onto medium containing glyphosate (100 mg/l). After two 14-day passages, embryos were transferred onto medium containing successively greater glyphosate concentrations, up to 540 mg/l, and regeneration was carried out as previously described (Held *et al.*, 2004).

The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-resistant form of the native maize EPSP synthase. Except for the introduced TIPS mutations, the amino acid sequence of the double-mutated maize EPSPS (2mEPSPS) enzyme expressed in maize line HCEM485 is identical to the native wild-type maize EPSPS sequence reported by Gardiner *et al.*, 2004 (Figure 2). Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species.

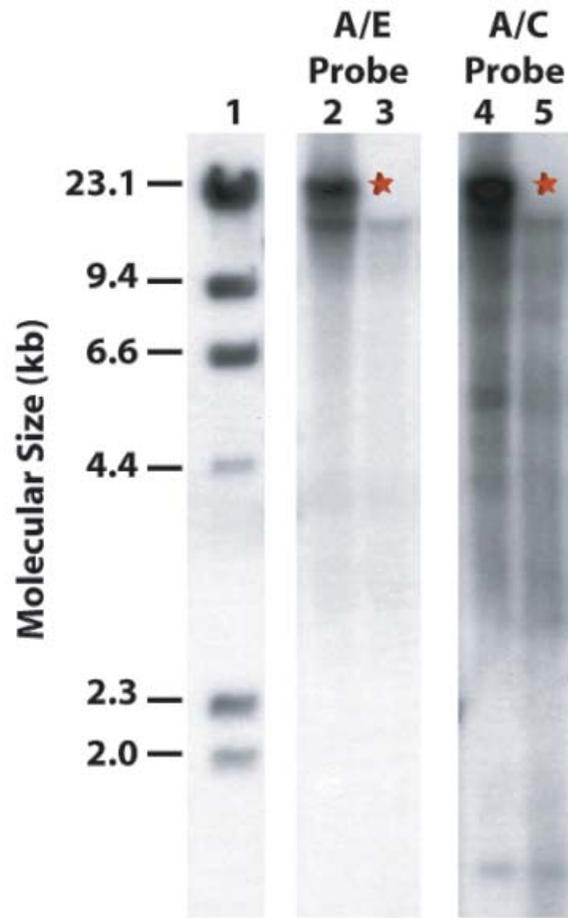
IV. GENETIC ANALYSIS OF MAIZE LINE HCEM485

IV.1 MOLECULAR CHARACTERIZATION

Southern analysis of HCEM485 maize DNA was performed in order to estimate the number of sites of insertion of the introduced DNA. Two probes were used that together spanned the entire 6.0 kb maize DNA fragment introduced into HCEM485. These probes were designated: a) A/C – obtained from a double digest of the pHCEM plasmid with *ClaI* and *Acc65I* (corresponding to positions 1–2346); and b) A/E – obtained from a double digest of the pHCEM plasmid with *Acc65I* and *EcoRV* (corresponding to positions 2347–6010). Probes (*ca.* 50 ng each) were labeled with 50 μ Ci of (α -³²P)-dCTP (3000 Ci/mmol) using a random labeling system (Rediprime™ II, Amersham Piscataway, NJ). Genomic DNA (7 μ g) isolated from HCEM485 and control Stine 963 maize was digested (37°C, overnight) with *HindIII* and restriction fragments were separated by agarose gel electrophoresis followed by transfer onto Hybond N+ nylon membrane. Southern hybridizations were performed according to standard procedures.

Southern analysis of HCEM485 genomic DNA using both the A/C (Figure 4, lane 4) and A/E (Figure 4, lane 2) probes following *HindIII* digestion indicated the presence of a single \geq 23 kb hybridizing fragment that was unique to HCEM485 (*i.e.*, not present in digests of control Stine 963 maize DNA). As there are no *HindIII* sites within the 6.0 kb maize DNA fragment introduced into HCEM485 and based on results from Southern analyses using other restriction endonucleases, it is postulated that multiple copies of the 6.0 kb fragment, approximately 4, were inserted at a single site within the maize genome.

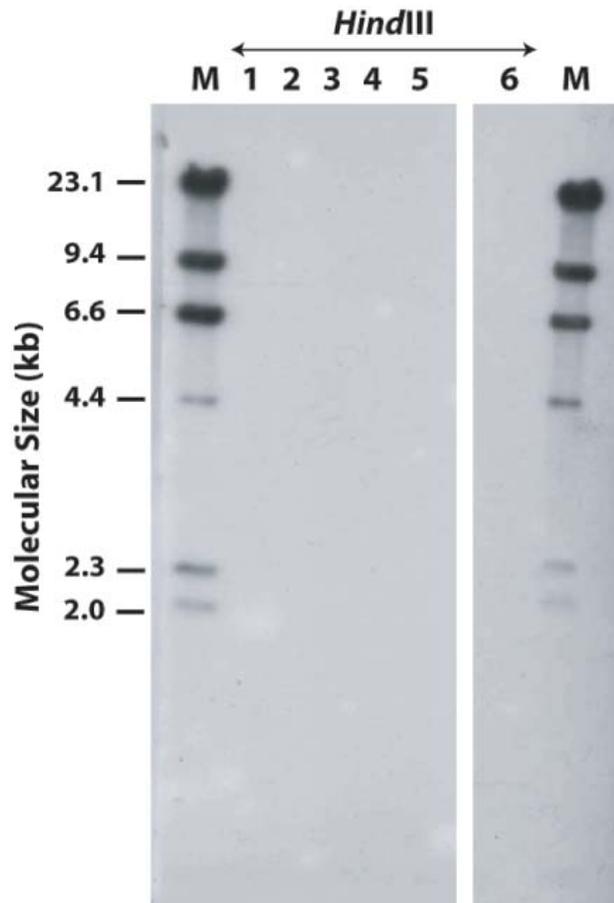
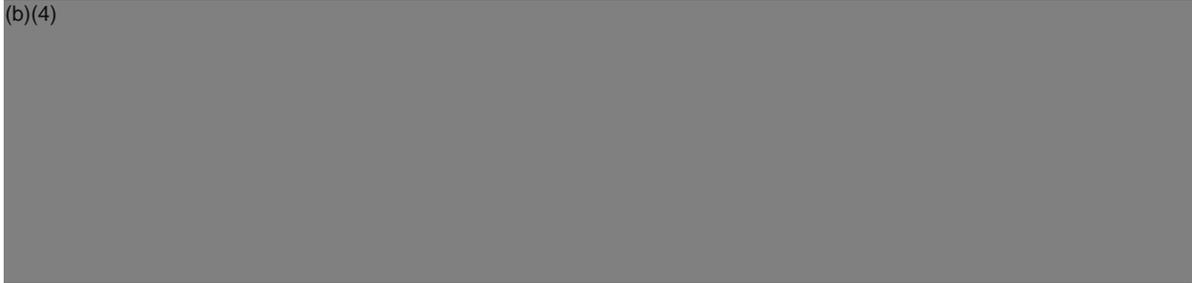
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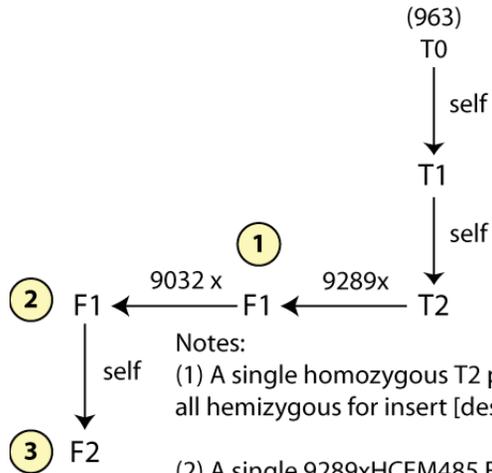
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Line HCEM485



Notes:

(1) A single homozygous T2 plant used in cross with line 9289, yielding F1 hybrids all hemizygous for insert [designated: 9289xHCEM485 F1].

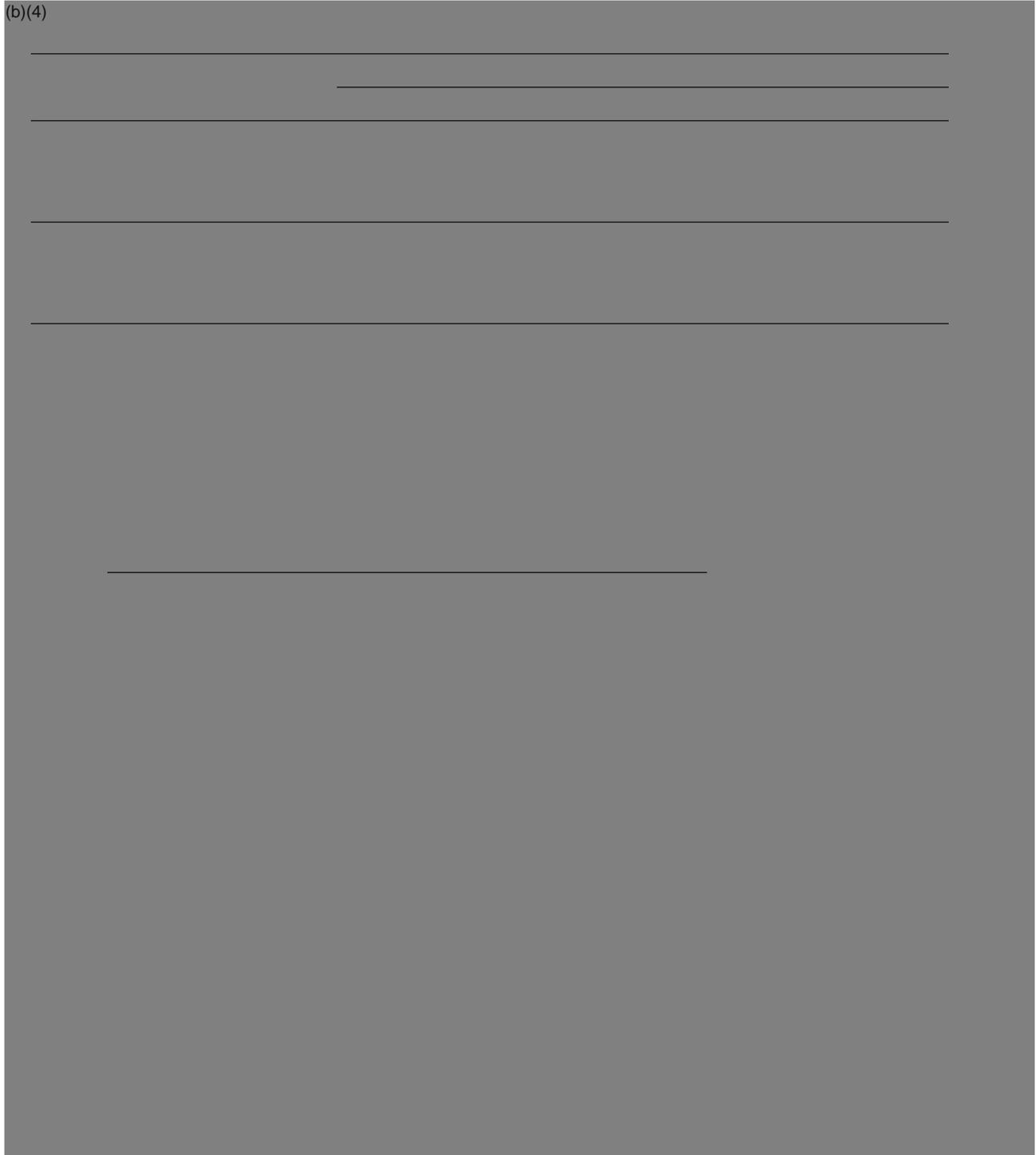
(2) A single 9289xHCEM485 F1 plant is crossed with line 9032, yielding F1 hybrids that should segregate 1:1 for the glyphosate tolerance trait [designated: (9289xHCEM485)9032 F1].

(3) F2 generation produced by selfing a single trait-positive plant from the preceding F1 generation. Progeny F2 plants should segregate 3:1 for the glyphosate-tolerance trait [designated: (9289xHCEM485)9032 S1F2].

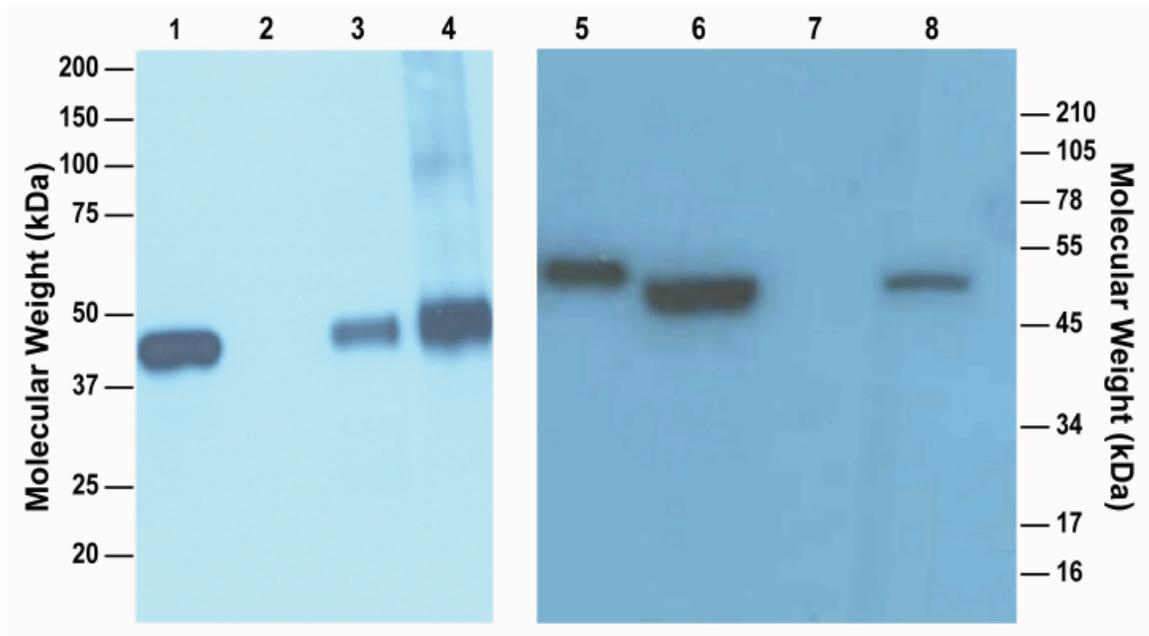
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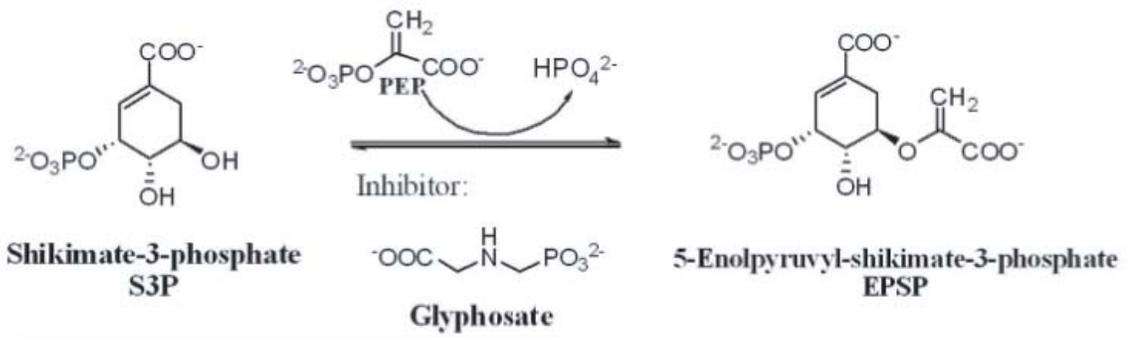


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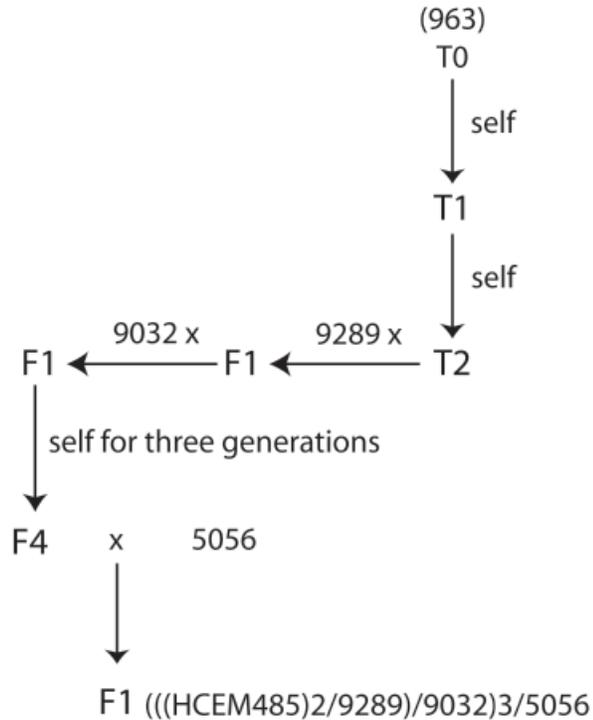




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Event HCEM485

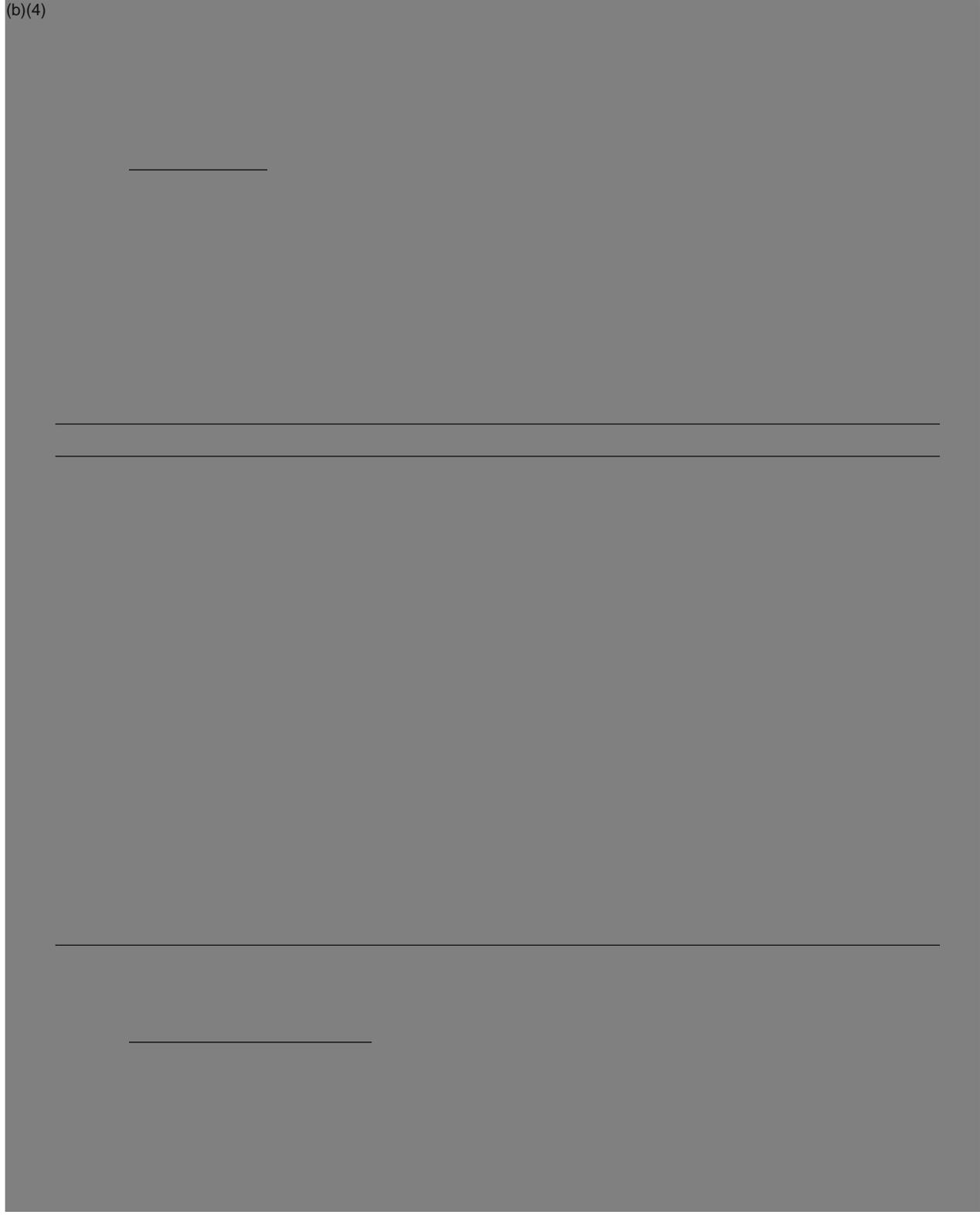


2007 Field Trials

HCEM485 Hybrid (((HCEM485)2/9289)/9032)3/5056 (trait positive)
Control Hybrid 9289/5056 (trait negative)
Control Hybrid 9032/5056 (trait negative)
Control Hybrid 963/5056 (trait negative)

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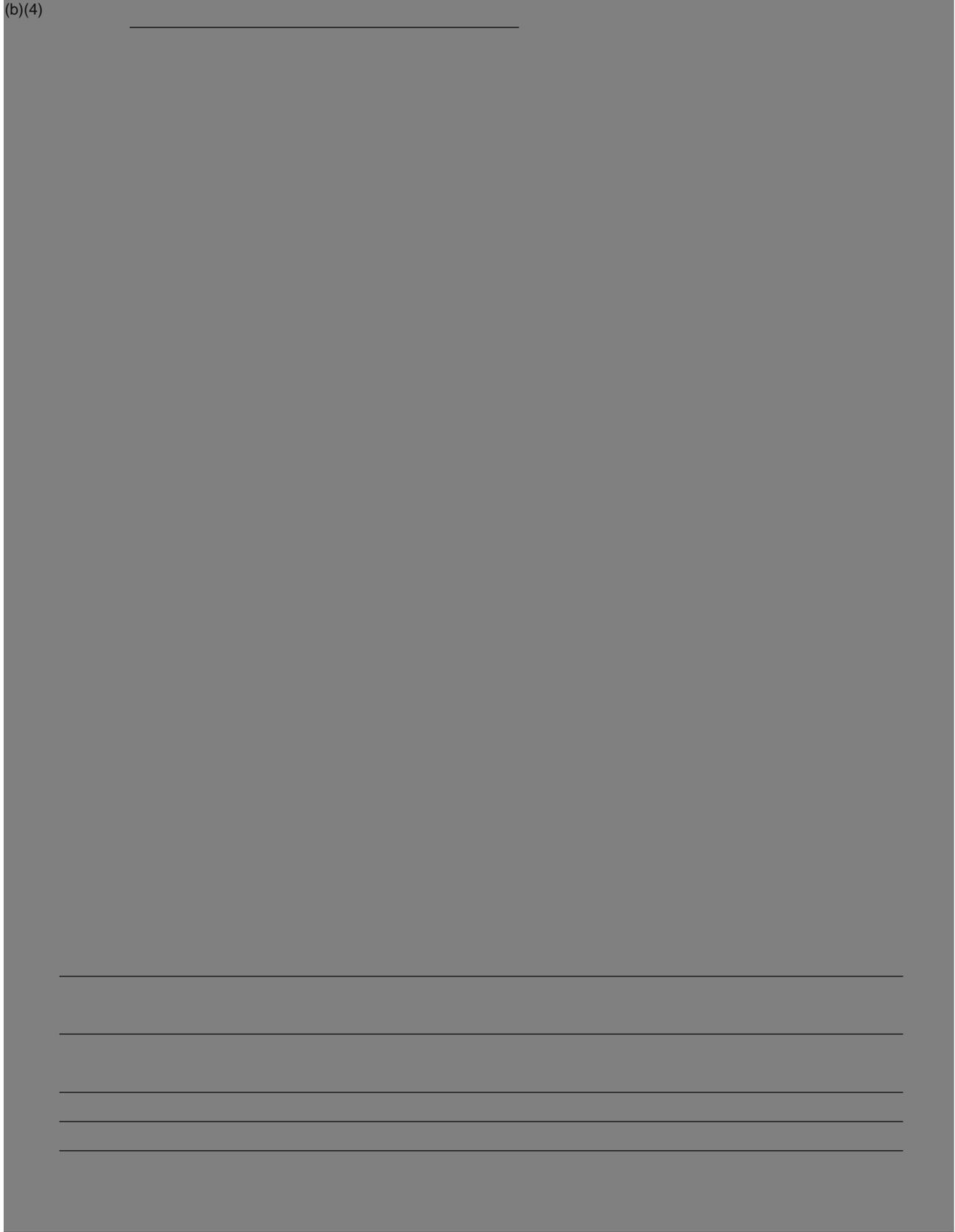
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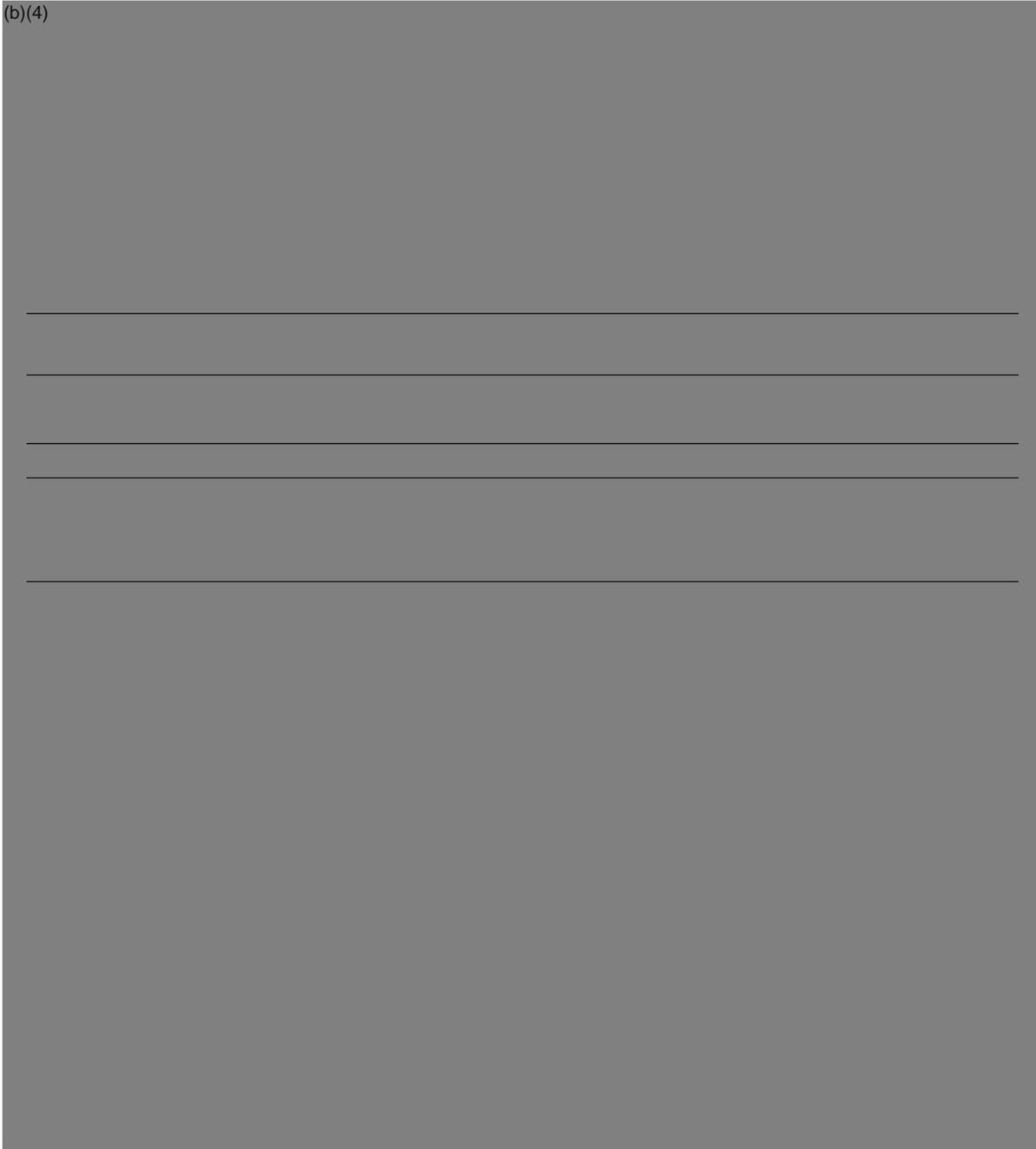
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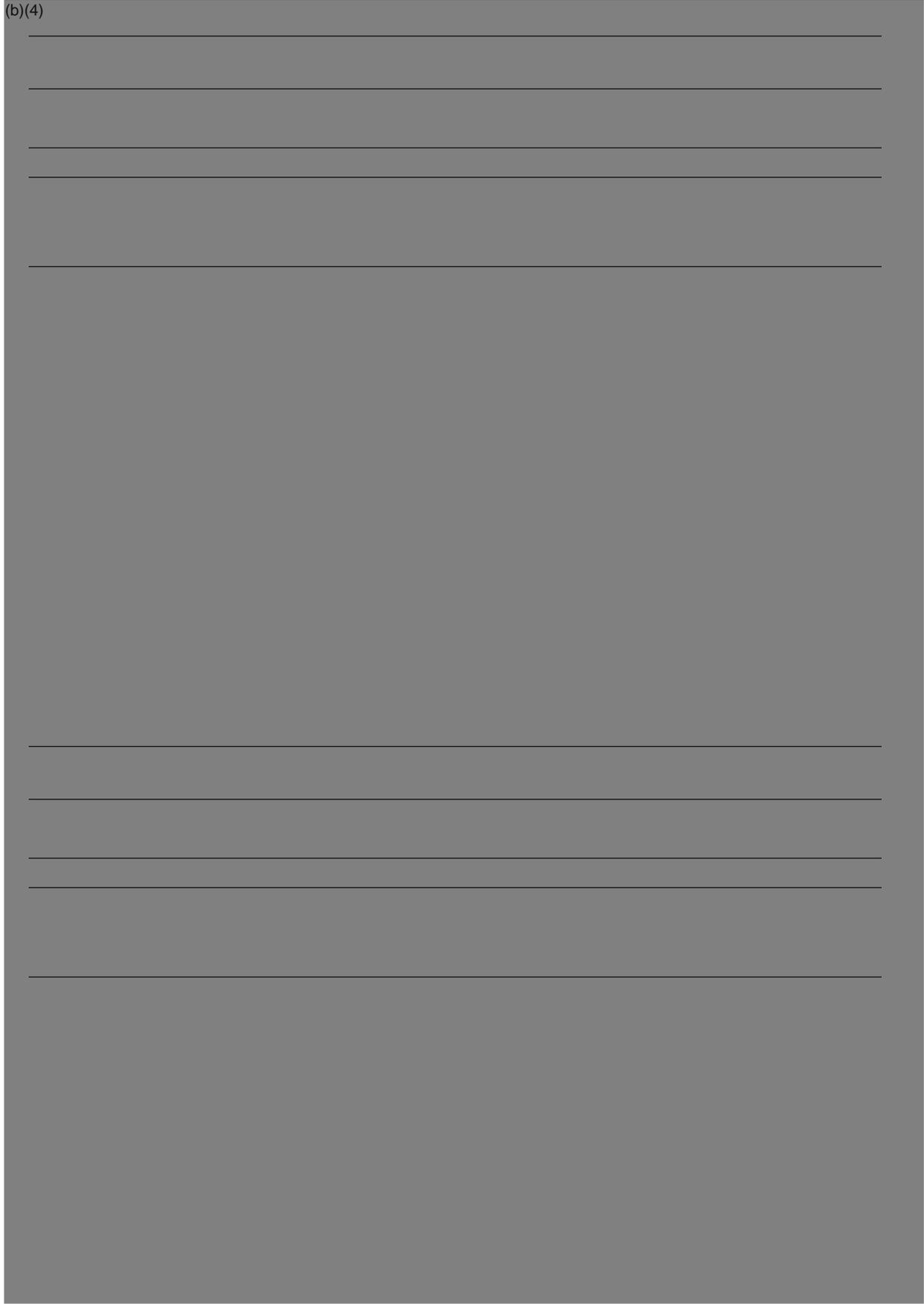


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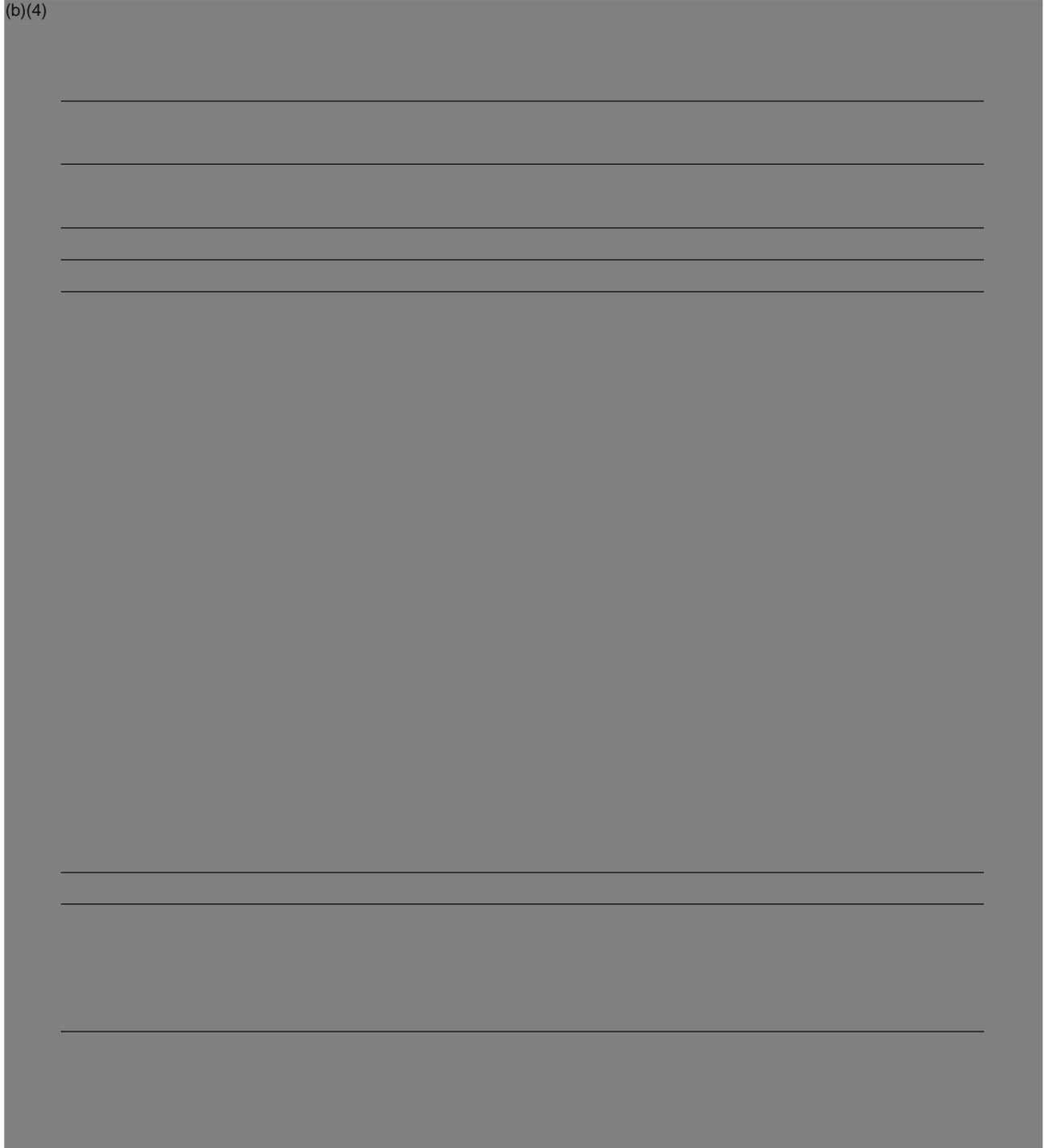


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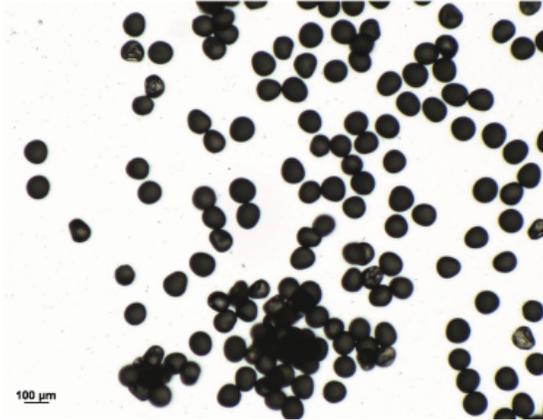


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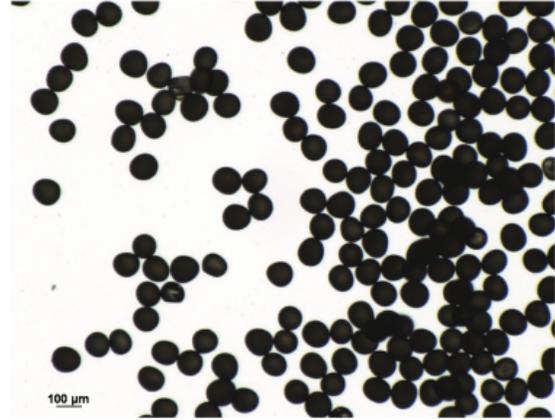


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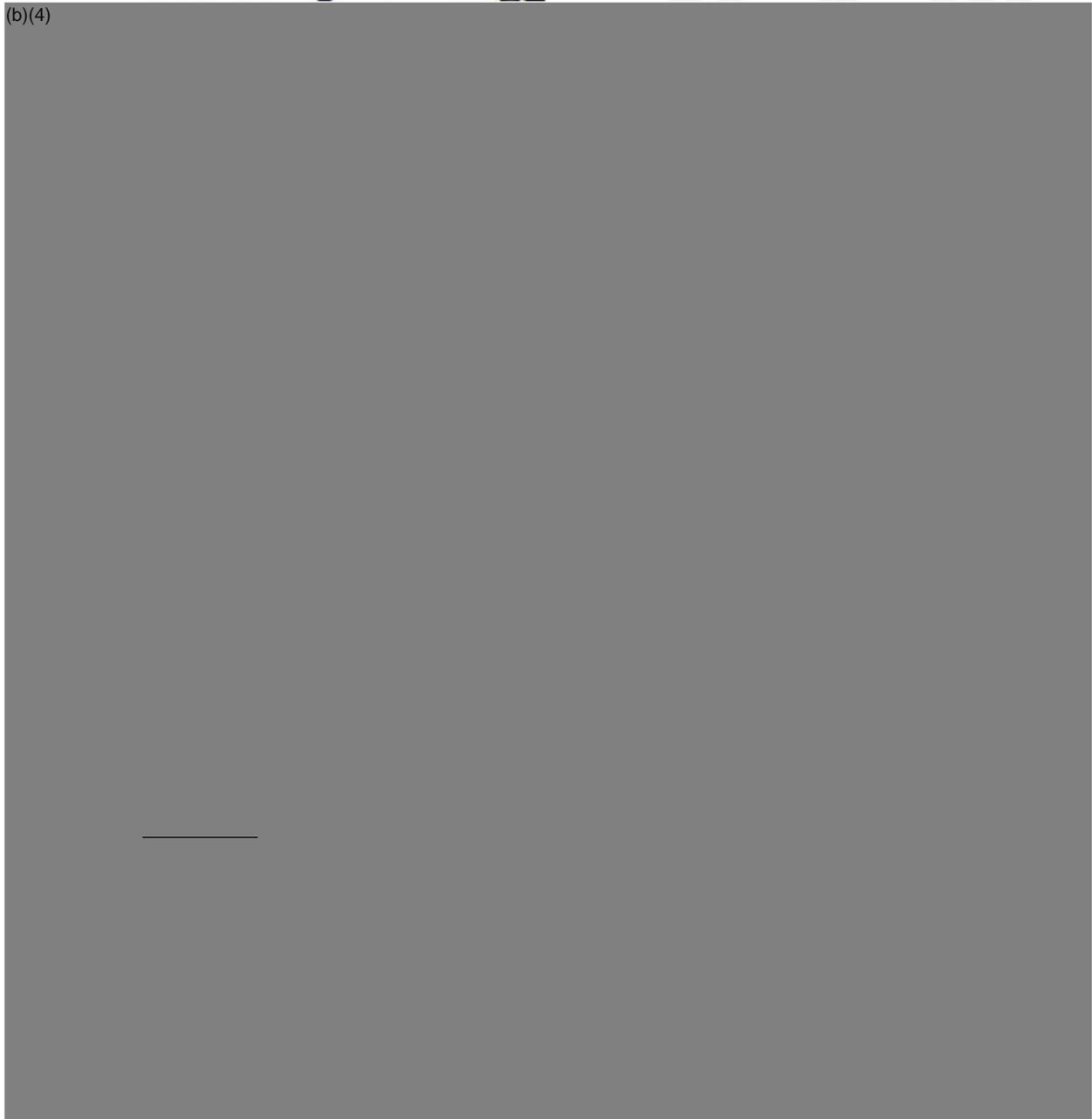
Control 9289 x 5056 Plant (80X Magnification)



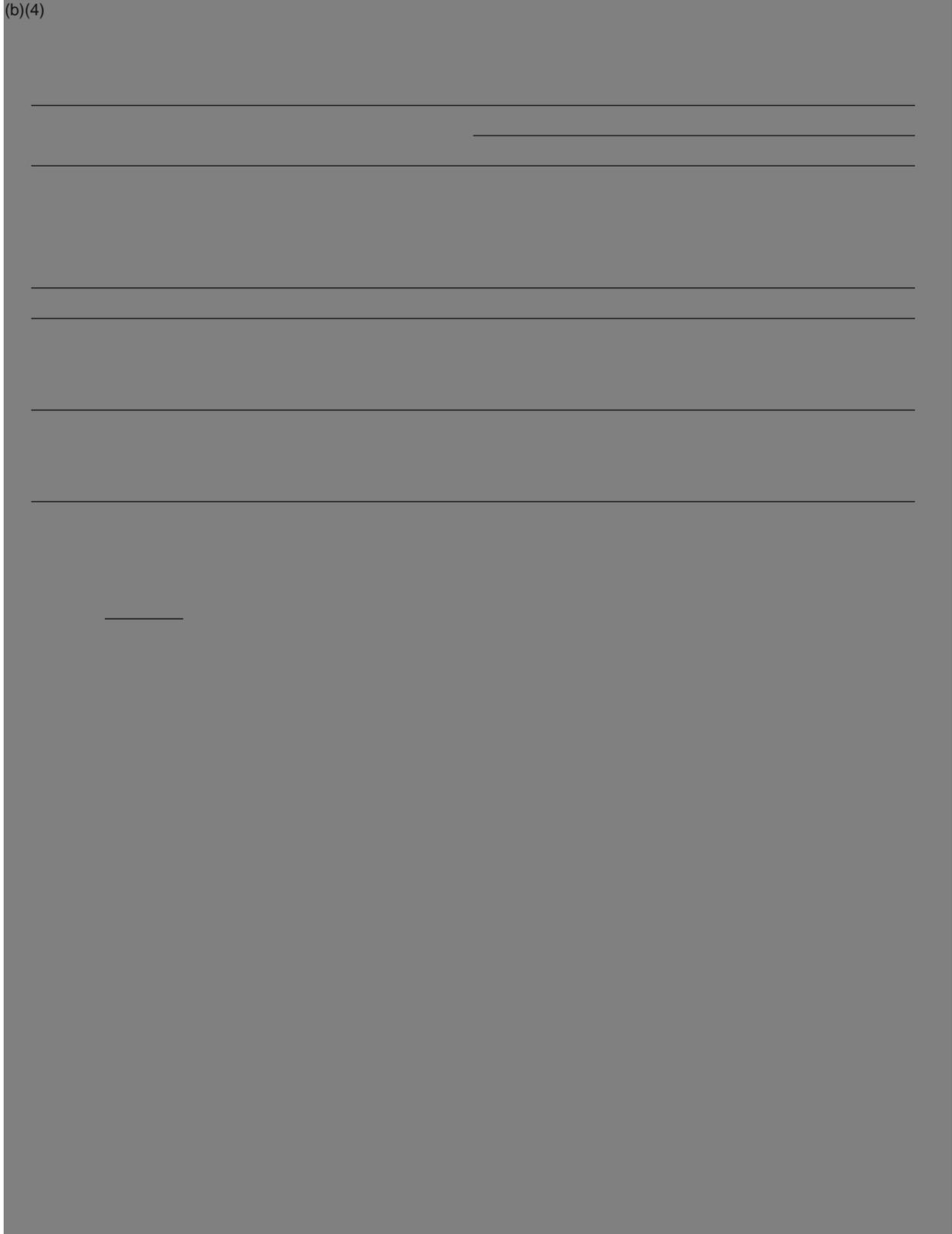
HCEM485 Plant (80X Magnification)



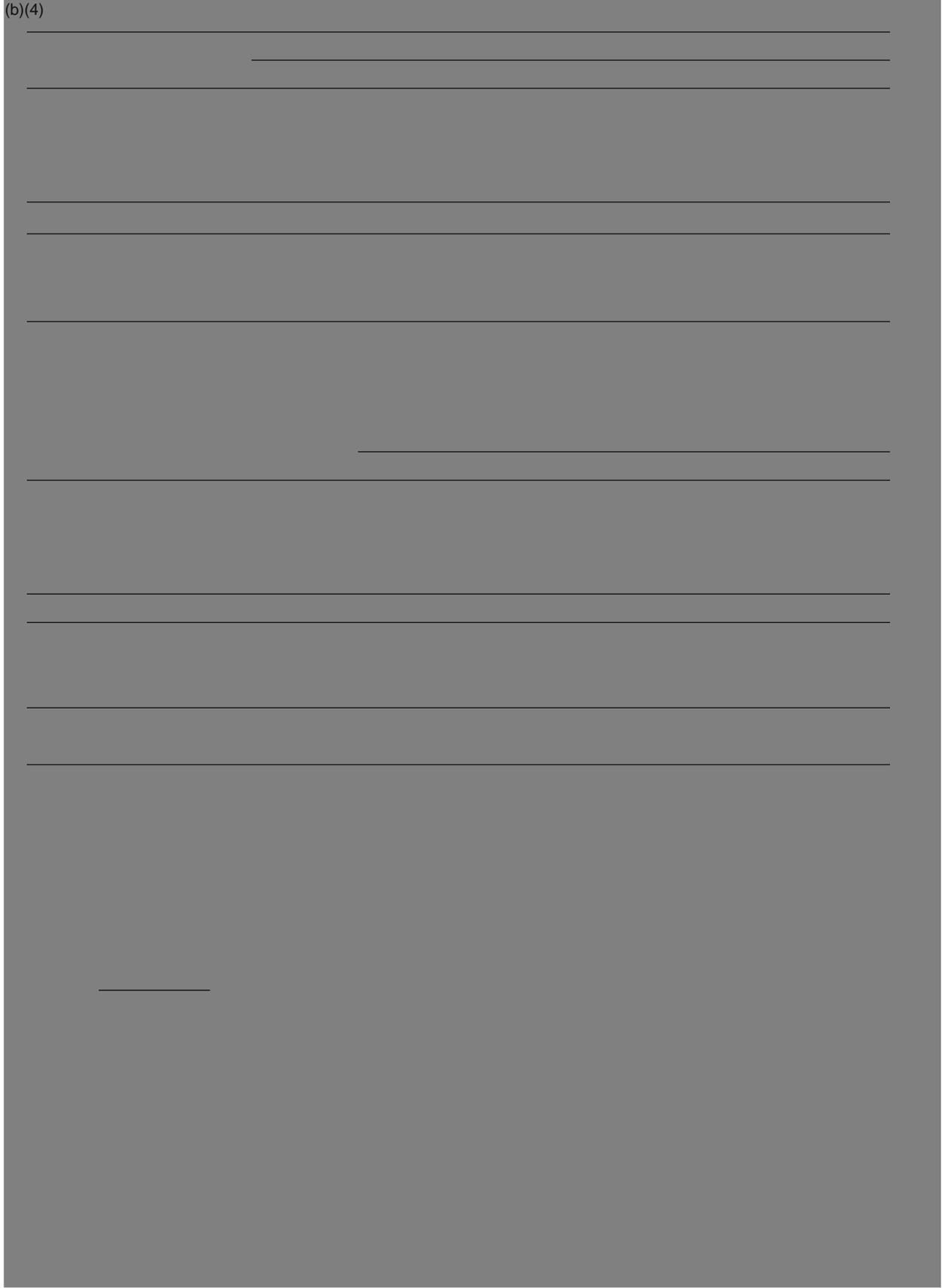
(b)(4)



(b)(4)



(b)(4)

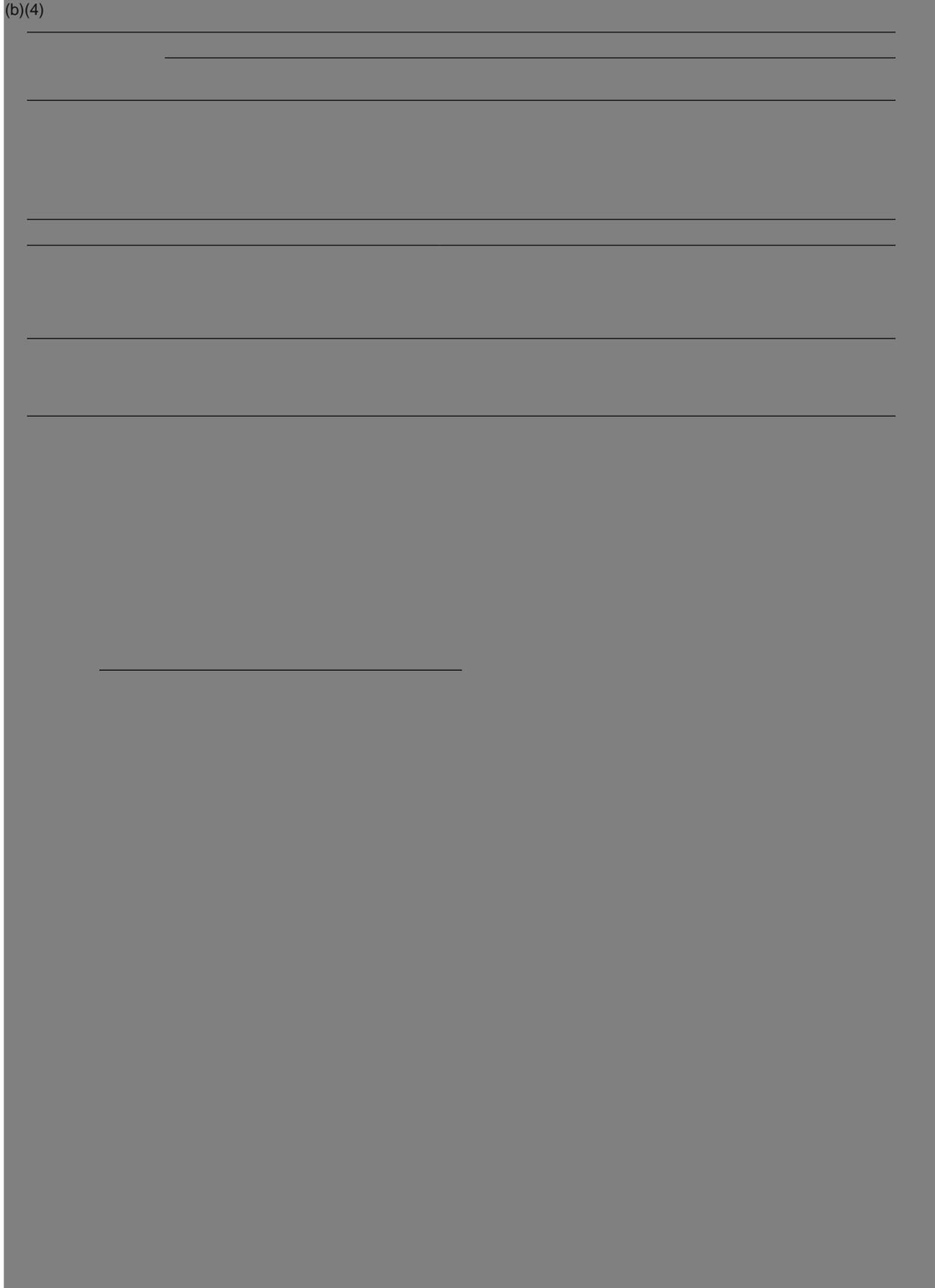


(b)(4)

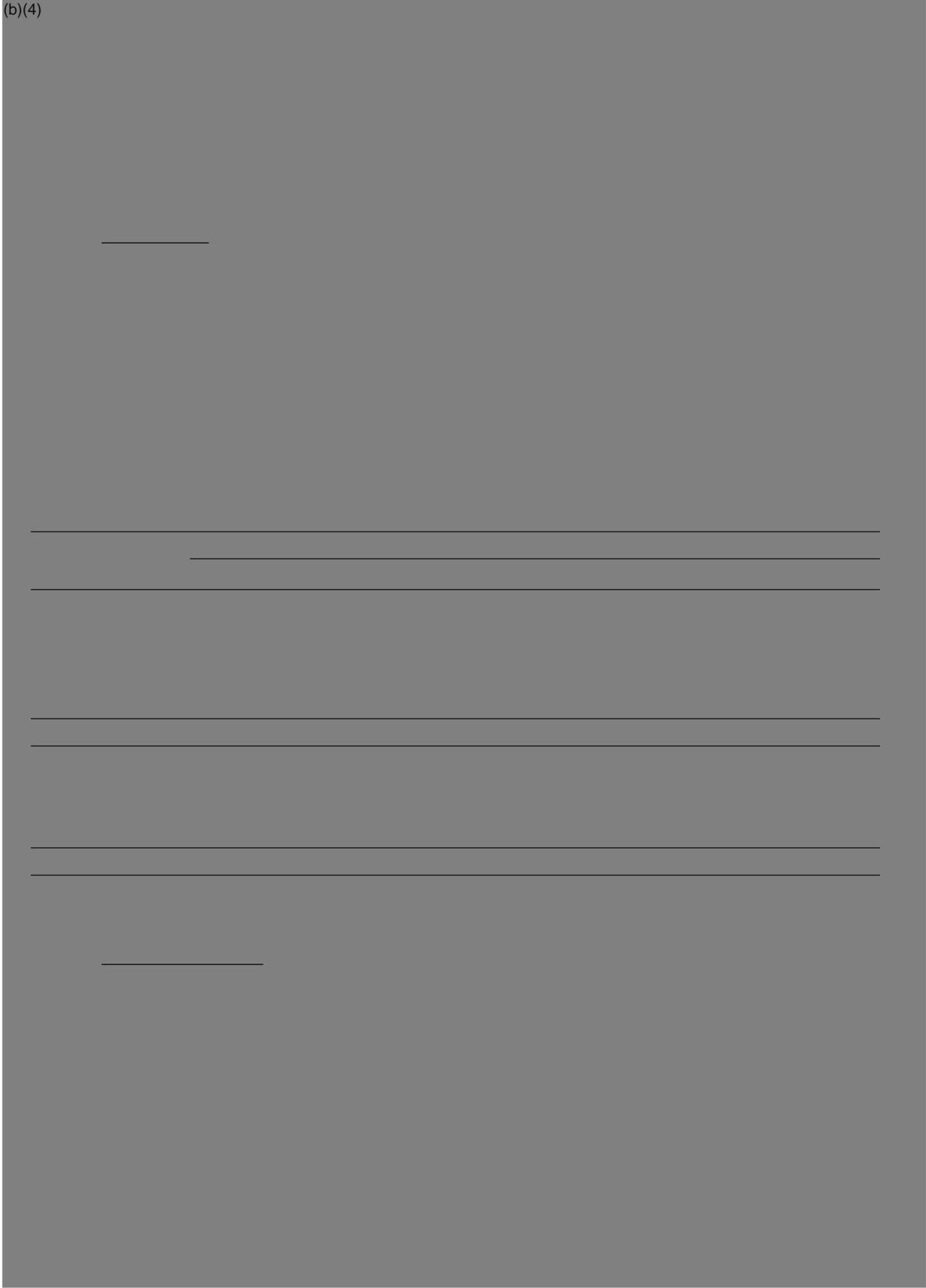


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VI. ENVIRONMENTAL IMPACT OF INTRODUCTION OF MAIZE LINE HCEM485

There are no changes from Section VI of the previously approved petition 97-099-01p in terms of the description of glyphosate herbicide, current uses of maize herbicides, weediness potential of glyphosate tolerant maize, cross pollination to wild and cultivated related species and transfer of genetic material to species to which maize cannot interbreed (*e.g.*, horizontal gene transfer). There is no expectation that cultivation of maize line HCEM485 would have any environmental effects different from the cultivation of the antecedent organism, GA21, or other maize lines exhibiting glyphosate tolerance that have also been deregulated by USDA-APHIS (*e.g.*, NK603; MON 88017; and MON 802).

VII. ADVERSE CONSEQUENCES OF INTRODUCTION

Stine Seed Farm knows of no study results or observations associated with maize line HCEM485 that would be anticipated to result in adverse environmental consequences from its introduction. Therefore, on the basis of the substantial phenotypic equivalence between maize line HCEM485 and the antecedent organism, GA21, Stine Seed Farm requests that an extension of nonregulated status be granted to maize line HCEM485.

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IX. APPENDIX 1: HCEM NUCLEOTIDE SEQUENCE

LOCUS HCEM 6010 bp DNA linear
 DEFINITION *Zea mays* fragment containing EPSPS encoding sequences, following site-directed mutagenesis
 ACCESSION Not-Specified.
 VERSION Not-Specified.
 KEYWORDS
 SOURCE *Zea mays*
 ORGANISM

FEATURES
 source Location/Qualifiers
 1..6010
 5' UTR
 1..1867
 /note="epsps regulatory sequences"
 transit_peptide 1868..2041
 /gene="CTP"
 CDS 1868..2167
 /gene="epsps exon 1"
 CDS 2696..2944
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 CDS 3044..3193
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 CDS 3467..3679
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 CDS 4189..4302
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 CDS 4756..4818
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 CDS 4928..5146
 /gene="epsps exon 8"
 gene 2042..5146
 /gene="2mEPSPS"
 3' UTR 5147..6010

BASE COUNT 1581 a 1375 c 1288 g 1766 t
 ORIGIN

```

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241 cacaggacac gcacaggaca cgcaaacagt ttcagactca tgcacacgca catcagtttc
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481 gatggcagta tacagacggc aaagttaatg ccggtgtaca tgcccttaga ctcttccgctc
541 acaactcac ttagatthttt acaacggaac ataaggttcg cttgcagact tacatataag
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661 aatattagta cacaatatta aataagaacg aacaatacat atattatcat tgatcttagt
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901 ctggctact tgmetaattg gtagtgacat ttgggaccgt ctgacacga cmetaaata
961 gtagtctaaa acatagctcg acacgatgcc tmetaaagat acgacaaagc acaacacgat
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1081 ataataatctt aatggttatt ttatgttcca ataattttca tctctcaaa aaaatgttat
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No CBI
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6001 cgttgatata  
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**X. APPENDIX 2: USDA APHIS RELEASE NOTIFICATIONS
RELEVANT TO THE FIELD TESTING OF MAIZE LINE HCEM485**

USDA #	Internal ID	County and State of Release
05-060-09n	SSF2005-006	Dallas, IA; Marshall, IA; Madison, IA; Lincoln, IL; Warren, IL; Vermillion, IL; Tipton, IN; Hamilton, IN; Boone, IN; Blue Earth, MN; Clinton, MO; Saline, MO; Valley, NE; Dodge, NE; Paulding, OH.
06-047-09n	SSF2006-001	Dallas, IA; Cass, IA; Logan, IL; Vermillion, IL; Tipton, IN; Hamilton, IN; Boone, IN; Lincoln, SD; Renville, MN; Valley, NE; Arkansas, AR; Paulding, OH.
07-046-110n	SSF2007-003	Dallas, IA; Tipton, IN; Hamilton, IN; Vermillion, IL; Logan, IL; Defiance, OH; Shiawassee, MI; Renville, MN; Kandiyohi, MN; Crittenden, AR; Lincoln, SD; Cedar, NE; Cass, IA; Warren, IL.
08-046-109n	SSD2008-003	Dallas, IA.

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Annex 1

(b)(4)



STUDY TITLE

(b)(4)

LABORATORY STUDY ID

(b)(4)

STUDY COMPLETED ON

30 November 2007

PERFORMING FACILITY

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

SUBMITTED BY

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

Summary

Agronomic and phenotypic characteristics of a HCEM485 maize hybrid and three control hybrids were evaluated in a series of field trials across 15 United States Corn Belt locations in 2007. HCEM485 maize produces a form of the maize 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme that was specifically modified through site-directed mutagenesis to confer tolerance to glyphosate-containing herbicides.

Up to 17 separate agronomic characteristics were assessed at each location, but not all traits were assessed at all locations. These agronomic traits covered a broad range of characteristics encompassing the entire life cycle of the maize plant and included data assessing germination and seedling emergence, growth habit, vegetative vigor, days to pollen shed, days to maturity, and yield parameters

Results of these trials suggest that there were no biologically significant unintended effects on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight, or disease susceptibility as a result of the genetic modification introduced into maize line HCEM485. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of outcrossing than unmodified maize.

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No CBI
Maize Line HCEM485
USDA Extension Petition

Statement of Good Laboratory Practices

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATORS:

(b)(6)

2-24-09

Date

2/24/09

Date

Feb. 24, 2009

Date

SUBMITTER/SPONSOR:

Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003

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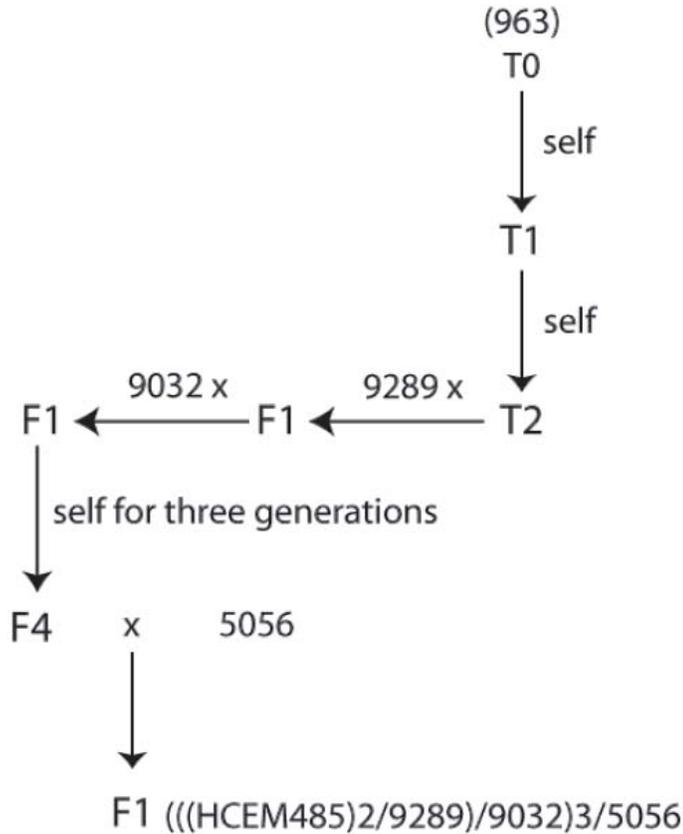
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Event HCEM485

**2007 Field Trials**

- HCEM485 Hybrid (((HCEM485)2/9289)/9032)3/5056 (trait positive)
- Control Hybrid 9289/5056 (trait negative)
- Control Hybrid 9032/5056 (trait negative)
- Control Hybrid 963/5056 (trait negative)

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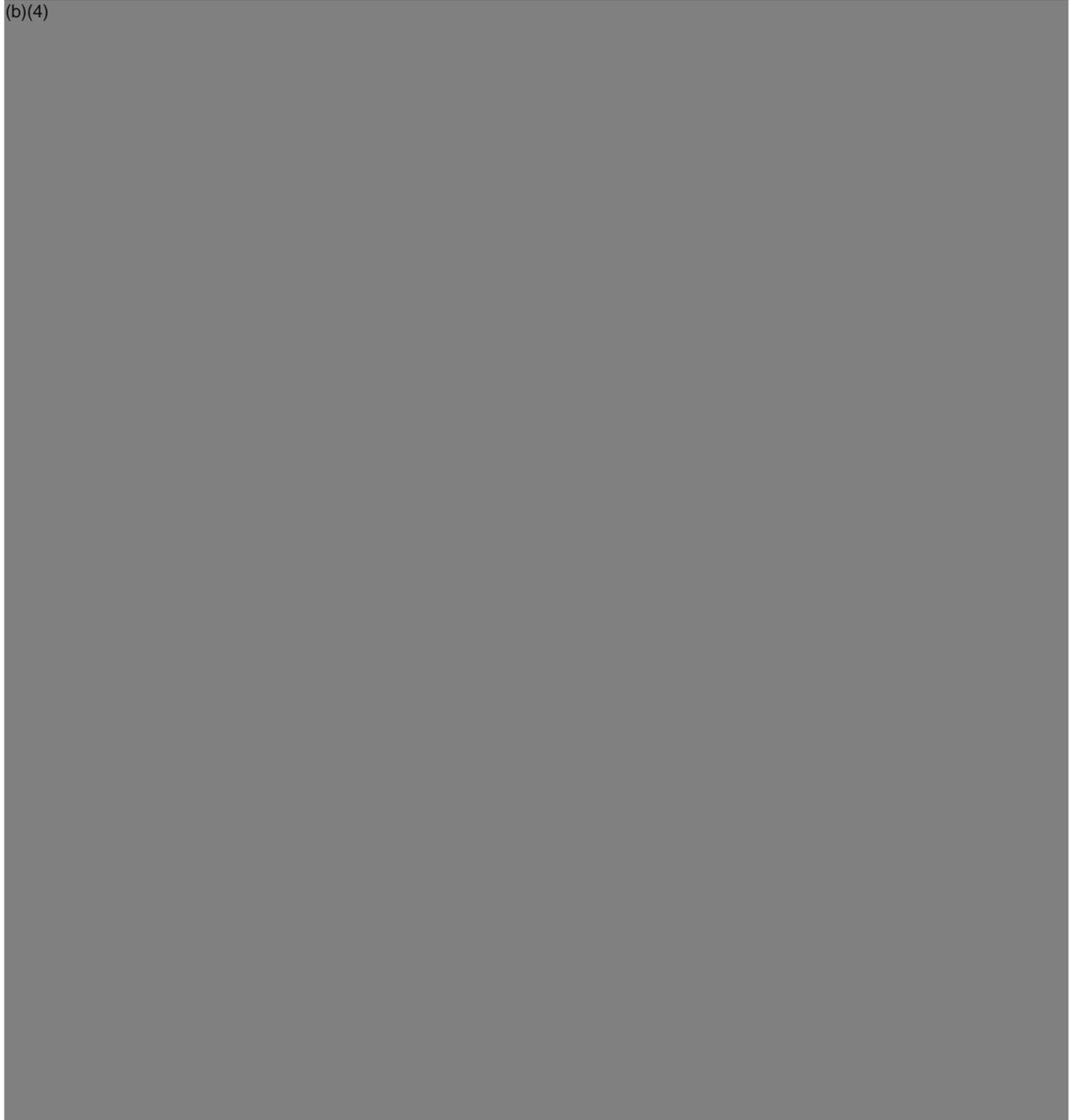
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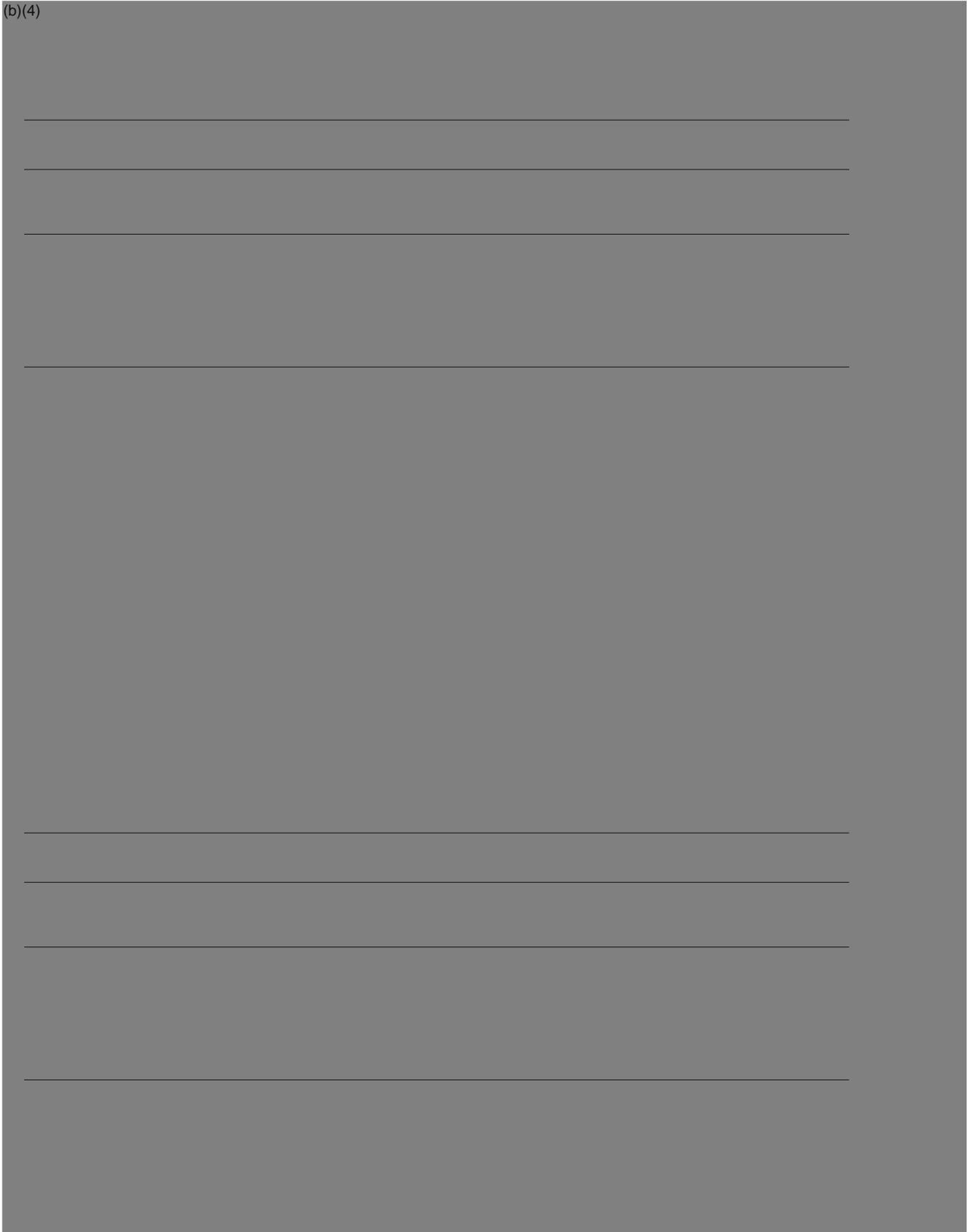
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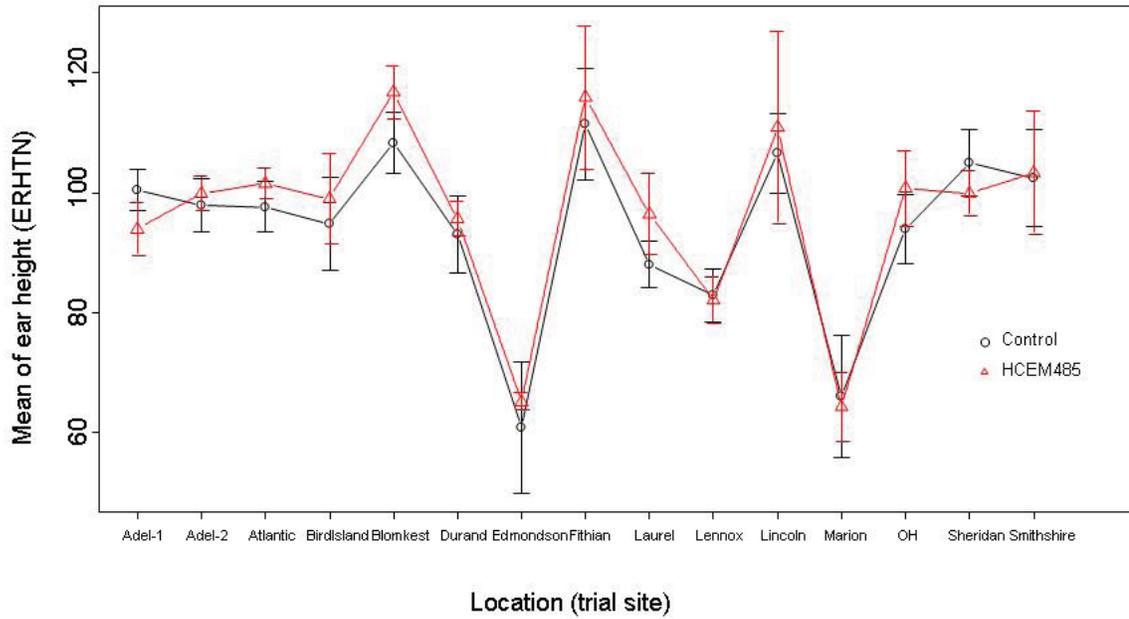
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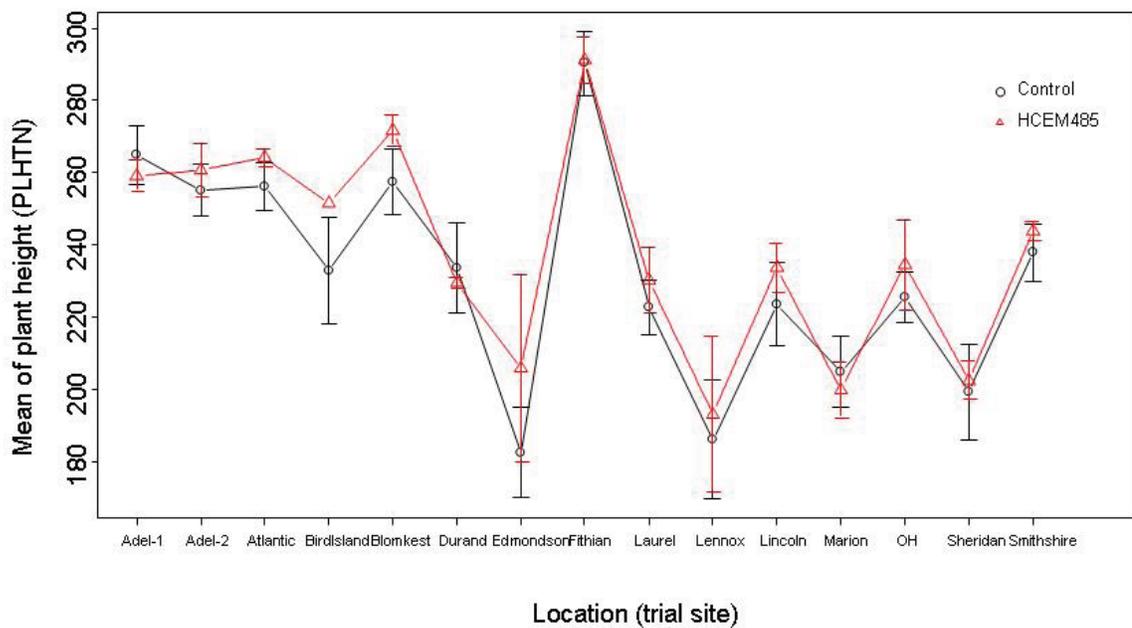
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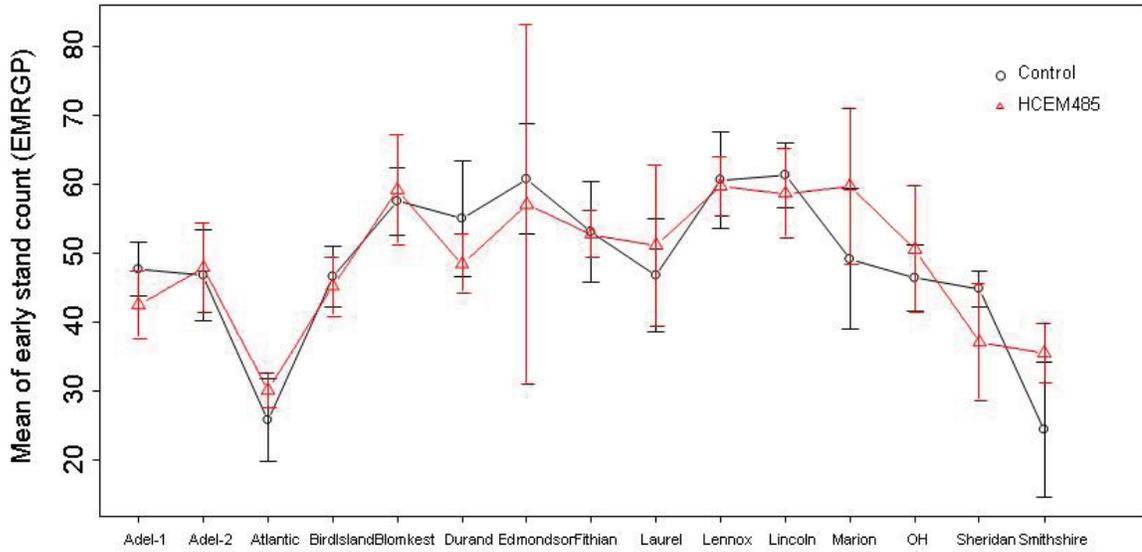
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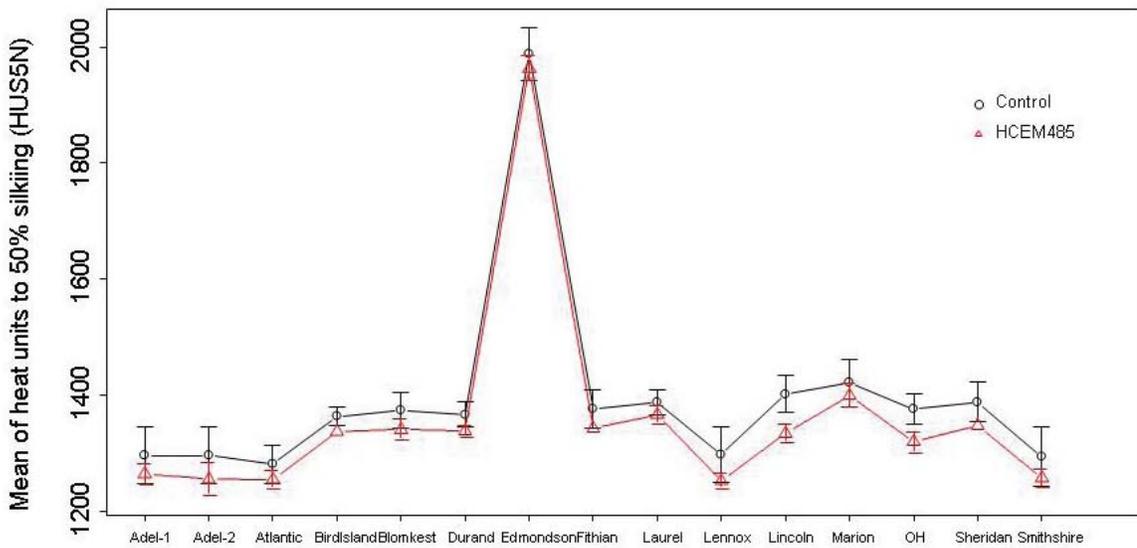
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Location (trial site)

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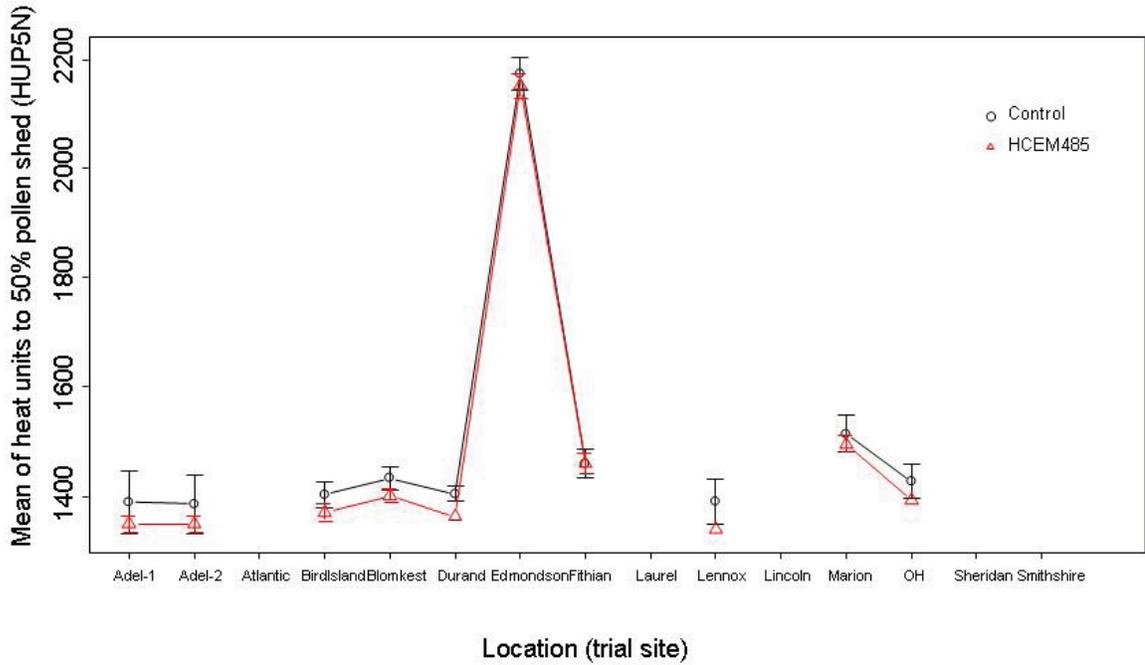


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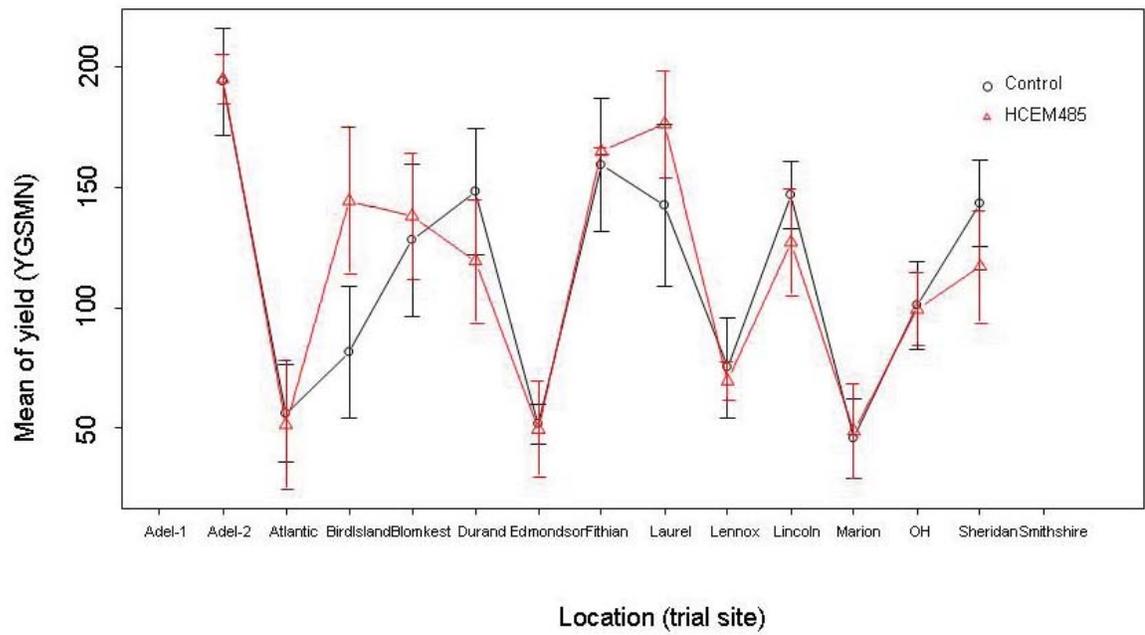
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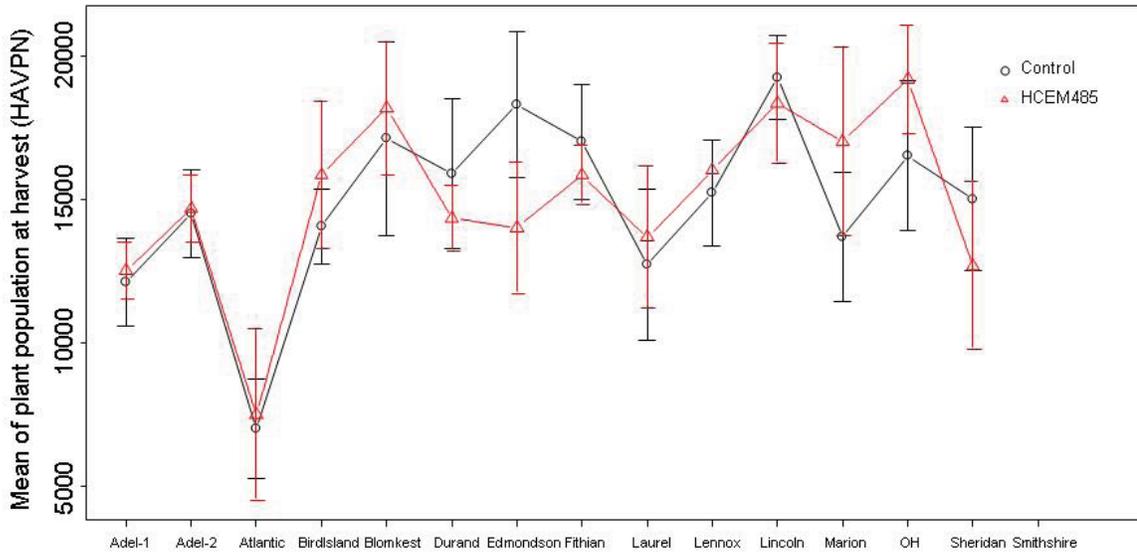
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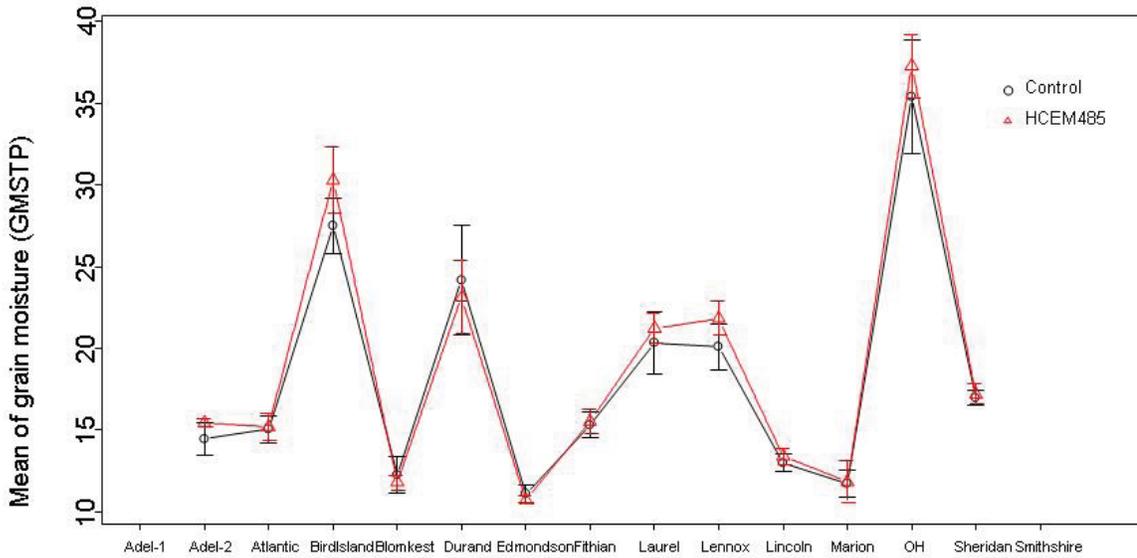
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Location (trial site)

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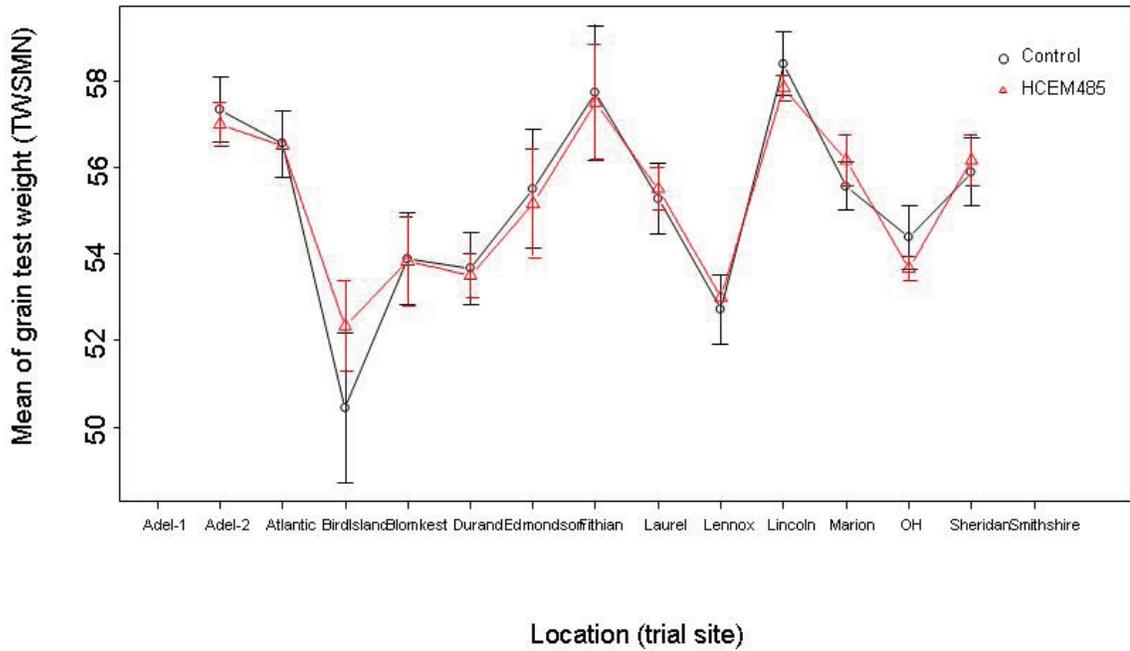


Location (trial site)

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8. APPENDIX B: HYBRID-BY-LOCATION MEANS

Location Code	Treatment	ERTLR (1-9 rating)	STKLR (1-9 rating)	RTLDR (1-9 rating)	LF CLR (1-9 rating)	EMRGR (1-9 rating)
ADL1	HCEM485 hybrid	9.0	6.3	9.0	4.7	7.0
	Control hybrids	9.0	6.7	9.0	5.0	7.7
	Mean Difference	0.0	-0.3	0.0	-0.3	-0.7
ADL2	HCEM485 hybrid	9.0	8.3	9.0	5.0	7.0
	Control hybrids	9.0	8.6	9.0	5.0	7.6
	Mean Difference	0.0	-0.2	0.0	0.0	-0.6
ATL	HCEM485 hybrid	9.0	9.0	9.0	5.0	7.3
	Control hybrids	9.0	9.0	9.0	5.0	7.1
	Mean Difference	0.0	0.0	0.0	0.0	0.2
BIR	HCEM485 hybrid	9.0	8.7	9.0	5.0	8.0
	Control hybrids	9.0	7.9	9.0	5.0	7.1
	Mean Difference	0.0	0.8	0.0	0.0	0.9
BLO	HCEM485 hybrid	9.0	9.0	9.0	5.0	8.0
	Control hybrids	9.0	9.0	9.0	5.0	7.8
	Mean Difference	0.0	0.0	0.0	0.0	0.2
DUR	HCEM485 hybrid	9.0	6.3	9.0	5.0	7.3
	Control hybrids	9.0	6.7	9.0	4.9	7.4
	Mean Difference	0.0	-0.3	0.0	0.1	-0.1
EDM	HCEM485 hybrid	9.0	8.0	9.0	5.0	6.7
	Control hybrids	9.0	8.2	9.0	5.3	7.6
	Mean Difference	0.0	-0.2	0.0	-0.3	-0.9
FIT	HCEM485 hybrid	9.0	6.7	9.0	5.3	7.0
	Control hybrids	9.0	6.9	9.0	5.2	7.9
	Mean Difference	0.0	-0.2	0.0	0.1	-0.9
LAU	HCEM485 hybrid	8.0	9.0	9.0	5.0	7.7
	Control hybrids	8.0	9.0	9.0	5.0	7.2
	Mean Difference	0.0	0.0	0.0	0.0	0.4
LEN	HCEM485 hybrid	5.7	9.0	2.7	5.0	7.0
	Control hybrids	5.3	9.0	2.9	4.9	7.7
	Mean Difference	0.3	0.0	-0.2	0.1	-0.7
LIN	HCEM485 hybrid	9.0	8.3	9.0	5.0	7.0
	Control hybrids	9.0	7.8	9.0	4.9	7.9
	Mean Difference	0.0	0.6	0.0	0.1	-0.9
MAR	HCEM485 hybrid	9.0	8.0	9.0	5.3	7.3
	Control hybrids	9.0	8.3	9.0	4.5	7.7
	Mean Difference	0.0	-0.3	0.0	0.8	-0.3
OH	HCEM485 hybrid	9.0	8.7	9.0	3.7	6.3
	Control hybrids	9.0	8.7	9.0	3.7	6.4
	Mean Difference	0.0	0.0	0.0	0.0	-0.1
SHE	HCEM485 hybrid	9.0	9.0	9.0	5.0	7.7
	Control hybrids	9.0	8.6	9.0	5.4	8.0
	Mean Difference	0.0	0.4	0.0	-0.4	-0.3
SMI	HCEM485 hybrid	9.0	NA	NA	5.0	6.7
	Control hybrids	9.0	NA	NA	4.8	6.9
	Mean Difference	0.0	NA	NA	0.2	-0.2

Location Code	Treatment	EAGRR (1-9 rating)	ERHTN (cm)	PLHTN (cm)	EMRGP (%)	HUSSN (heat units)
ADL1	HCEM485 hybrid	8.67	93.58	259.08	42.47	1264.00
	Control hybrids	8.11	100.47	265.01	47.67	1296.33
	Mean Difference	0.56	-6.49	-5.93	-5.20	-32.33
ADL2	HCEM485 hybrid	8.00	99.51	260.77	47.85	1255.00
	Control hybrids	8.33	97.53	255.13	46.77	1296.33
	Mean Difference	-0.33	1.98	5.54	1.08	-41.33
ATL	HCEM485 hybrid	7.33	101.60	264.16	37.11	1254.50
	Control hybrids	7.33	97.65	256.26	25.81	1281.56
	Mean Difference	0.00	3.95	7.90	4.30	-27.06
PIR	HCEM485 hybrid	6.33	99.06	251.46	45.16	1337.00
	Control hybrids	5.00	94.83	232.83	46.59	1363.11
	Mean Difference	1.33	4.23	18.63	-1.43	-26.11
PLO	HCEM485 hybrid	6.67	116.84	271.78	59.14	1340.83
	Control hybrids	6.11	108.37	257.39	57.53	1373.39
	Mean Difference	0.56	8.47	14.39	1.61	-32.56
DUR	HCEM485 hybrid	8.33	95.67	229.45	48.39	1337.83
	Control hybrids	8.67	93.13	233.68	55.02	1365.94
	Mean Difference	-0.33	2.54	-4.23	-6.63	-28.11
EDM	HCEM485 hybrid	6.67	65.19	205.74	56.99	1963.00
	Control hybrids	7.44	60.56	182.32	67.75	1988.00
	Mean Difference	-0.78	4.23	23.42	-3.76	-25.00
FIT	HCEM485 hybrid	7.67	115.99	291.25	52.69	1343.00
	Control hybrids	7.78	111.48	290.41	53.05	1375.89
	Mean Difference	-0.11	4.51	0.85	-0.36	-32.89
LAU	HCEM485 hybrid	8.00	96.52	230.29	51.08	1366.17
	Control hybrids	7.44	88.05	222.67	46.77	1387.06
	Mean Difference	0.56	8.47	7.52	4.30	-20.89
LEN	HCEM485 hybrid	7.67	82.13	193.04	59.68	1252.17
	Control hybrids	8.11	82.57	185.98	67.57	1297.83
	Mean Difference	-0.44	-0.85	7.06	-0.90	-45.67
LIN	HCEM485 hybrid	7.67	110.91	233.68	58.60	1334.00
	Control hybrids	8.00	106.68	223.52	61.29	1401.33
	Mean Difference	-0.33	4.23	10.16	-2.69	-67.33
MAR	HCEM485 hybrid	7.67	64.25	199.81	59.68	1399.17
	Control hybrids	7.75	66.04	204.79	49.10	1421.69
	Mean Difference	-0.08	-1.69	-4.97	13.57	-22.52
OH	HCEM485 hybrid	7.67	100.75	234.53	57.54	1319.33
	Control hybrids	7.44	93.58	225.50	46.42	1375.72
	Mean Difference	0.22	6.77	9.03	4.12	-56.39
SHE	HCEM485 hybrid	7.67	99.51	202.35	37.10	1347.50
	Control hybrids	8.11	104.99	199.25	44.80	1387.06
	Mean Difference	-0.44	-5.08	3.10	-7.71	-39.56
SMI	HCEM485 hybrid	8.00	103.29	243.84	35.48	1257.00
	Control hybrids	7.56	102.45	237.91	24.37	1294.11
	Mean Difference	0.44	0.85	5.93	11.11	-37.11

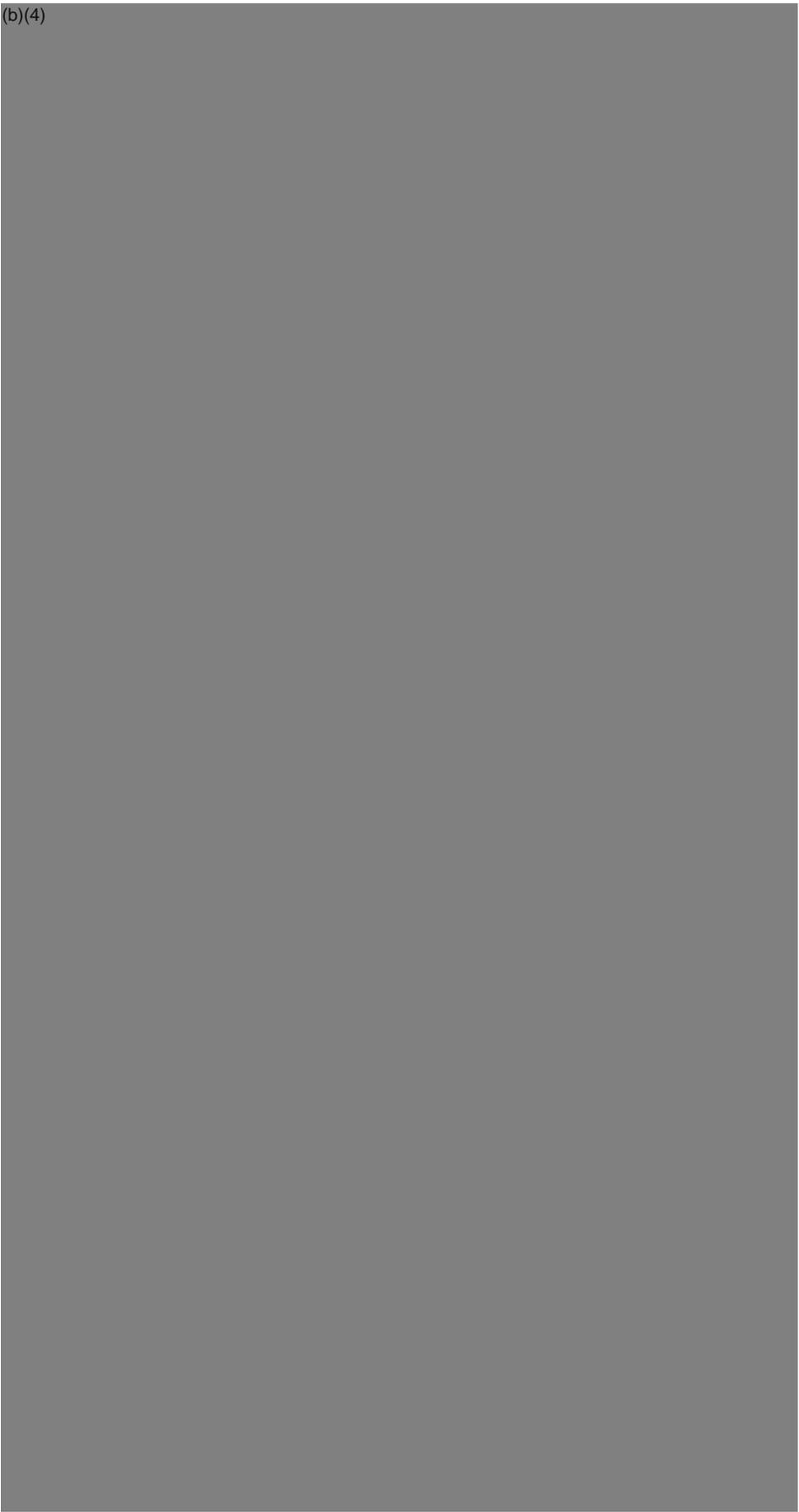
Location Code	Treatment	HUPSN (heat units)	BRRNP (%)	YGSMN (bu/acre)	HAVPN (plants/acre)	DROPP (%)
ADL1	HCEM485 hybrid	1350.00	0.00	NA	12500.0	0.00
	Control hybrids	1389.56	0.00	NA	12111.1	0.00
	Mean Difference	-39.56	0.00	NA	388.9	0.00
ADL2	HCEM485 hybrid	1350.00	0.00	194.98	14666.7	0.00
	Control hybrids	1386.33	0.00	193.97	14500.0	0.00
	Mean Difference	-36.33	0.00	1.01	166.7	0.00
ATL	HCEM485 hybrid	NA	0.00	51.20	7500.0	0.00
	Control hybrids	NA	0.00	55.82	7000.0	0.00
	Mean Difference	NA	0.00	-4.62	500.0	0.00
BIR	HCEM485 hybrid	1370.67	0.00	144.30	15833.3	0.00
	Control hybrids	1403.72	0.00	81.44	14055.6	0.00
	Mean Difference	-33.06	0.00	62.87	1777.8	0.00
BLO	HCEM485 hybrid	1401.67	0.00	137.99	18166.7	0.00
	Control hybrids	1433.67	0.00	128.18	17111.1	0.00
	Mean Difference	-32.00	0.00	9.81	1055.6	0.00
DUR	HCEM485 hybrid	1363.00	0.00	119.39	14333.3	0.00
	Control hybrids	1404.78	0.00	148.13	15888.9	0.00
	Mean Difference	-41.78	0.00	-28.74	-1555.6	0.00
EDM	HCEM485 hybrid	2151.83	0.00	49.26	14000.0	0.00
	Control hybrids	2173.89	0.00	51.47	18277.8	0.00
	Mean Difference	-22.06	0.00	-2.21	-4277.8	0.00
FIT	HCEM485 hybrid	1460.33	1.11	164.99	15833.3	0.00
	Control hybrids	1460.11	0.63	159.28	17000.0	0.00
	Mean Difference	0.22	0.49	5.71	-1166.7	0.00
LAU	HCEM485 hybrid	NA	0.00	176.32	13666.7	0.00
	Control hybrids	NA	0.00	142.52	12722.2	0.00
	Mean Difference	NA	0.00	33.80	944.4	0.00
LEN	HCEM485 hybrid	1340.50	0.00	69.46	16000.0	0.00
	Control hybrids	1390.89	0.00	75.07	15222.2	0.00
	Mean Difference	-50.39	0.00	-5.60	777.8	0.00
LIN	HCEM485 hybrid	NA	0.00	127.07	18333.3	0.88
	Control hybrids	NA	0.00	146.95	19222.2	0.60
	Mean Difference	NA	0.00	-19.88	-888.9	0.28
MAR	HCEM485 hybrid	1495.67	0.00	48.42	17000.0	0.00
	Control hybrids	1514.81	0.00	45.56	13687.5	0.00
	Mean Difference	-19.15	0.00	2.86	3312.5	0.00
OH	HCEM485 hybrid	1393.00	9.32	99.33	19166.7	0.00
	Control hybrids	1427.67	4.25	100.88	16500.0	0.00
	Mean Difference	-34.67	5.07	-1.55	2666.7	0.00
SHE	HCEM485 hybrid	NA	0.00	117.01	12666.7	0.00
	Control hybrids	NA	0.00	143.43	15000.0	0.00
	Mean Difference	NA	0.00	-26.43	-2333.3	0.00
SMI	HCEM485 hybrid	NA	NA	NA	NA	NA
	Control hybrids	NA	NA	NA	NA	NA
	Mean Difference	NA	NA	NA	NA	NA

Location Code	Treatment	GMSTP (%)	TWSMN (lb/ha)
ADL1	HCEM485 hybrid	NA	NA
	Control hybrids	NA	0.00
	Mean Difference	NA	NA
ADL2	HCEM485 hybrid	15.43	57.00
	Control hybrids	14.44	57.33
	Mean Difference	0.99	-0.33
ATL	HCEM485 hybrid	15.20	56.50
	Control hybrids	15.06	56.56
	Mean Difference	0.14	-0.06
BIR	HCEM485 hybrid	30.30	52.33
	Control hybrids	27.50	50.44
	Mean Difference	2.80	1.89
BLO	HCEM485 hybrid	11.80	53.83
	Control hybrids	12.24	53.89
	Mean Difference	-0.44	-0.06
DUR	HCEM485 hybrid	23.13	53.50
	Control hybrids	24.17	53.67
	Mean Difference	-1.03	-0.17
EDM	HCEM485 hybrid	10.70	55.17
	Control hybrids	11.10	55.50
	Mean Difference	-0.40	-0.33
FIT	HCEM485 hybrid	15.53	57.50
	Control hybrids	15.30	57.72
	Mean Difference	0.23	-0.22
LAU	HCEM485 hybrid	21.23	55.50
	Control hybrids	20.32	55.28
	Mean Difference	0.91	0.22
LEN	HCEM485 hybrid	21.83	53.00
	Control hybrids	20.10	52.72
	Mean Difference	1.73	0.28
LIN	HCEM485 hybrid	13.40	57.83
	Control hybrids	12.99	58.39
	Mean Difference	0.41	-0.56
MAR	HCEM485 hybrid	11.83	56.17
	Control hybrids	11.71	55.56
	Mean Difference	0.12	0.60
OH	HCEM485 hybrid	37.30	53.67
	Control hybrids	35.40	54.39
	Mean Difference	1.90	-0.72
SHE	HCEM485 hybrid	17.20	56.17
	Control hybrids	16.98	55.89
	Mean Difference	0.22	0.28
SMI	HCEM485 hybrid	NA	NA
	Control hybrids	NA	NA
	Mean Difference	NA	NA

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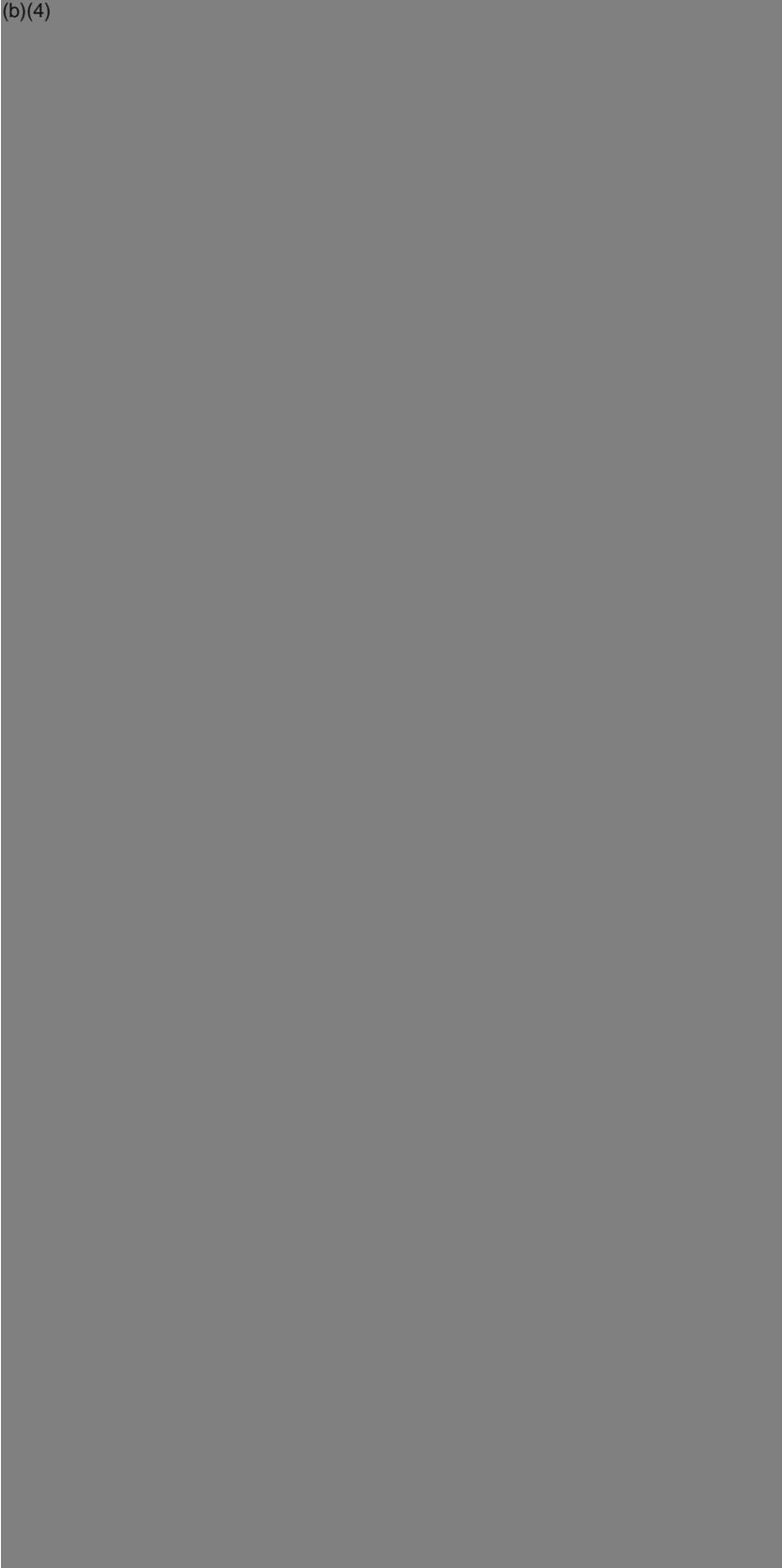


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STUDY TITLE



LABORATORY STUDY ID

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STUDY COMPLETED ON

15 October 2007

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SUBMITTED BY

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

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Summary

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USDA Extension Petition

Statement of Good Laboratory Practices

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATORS:

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02/24/2009

Date

Feb. 24, 2009

Date

SUBMITTER/SPONSOR:

Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003

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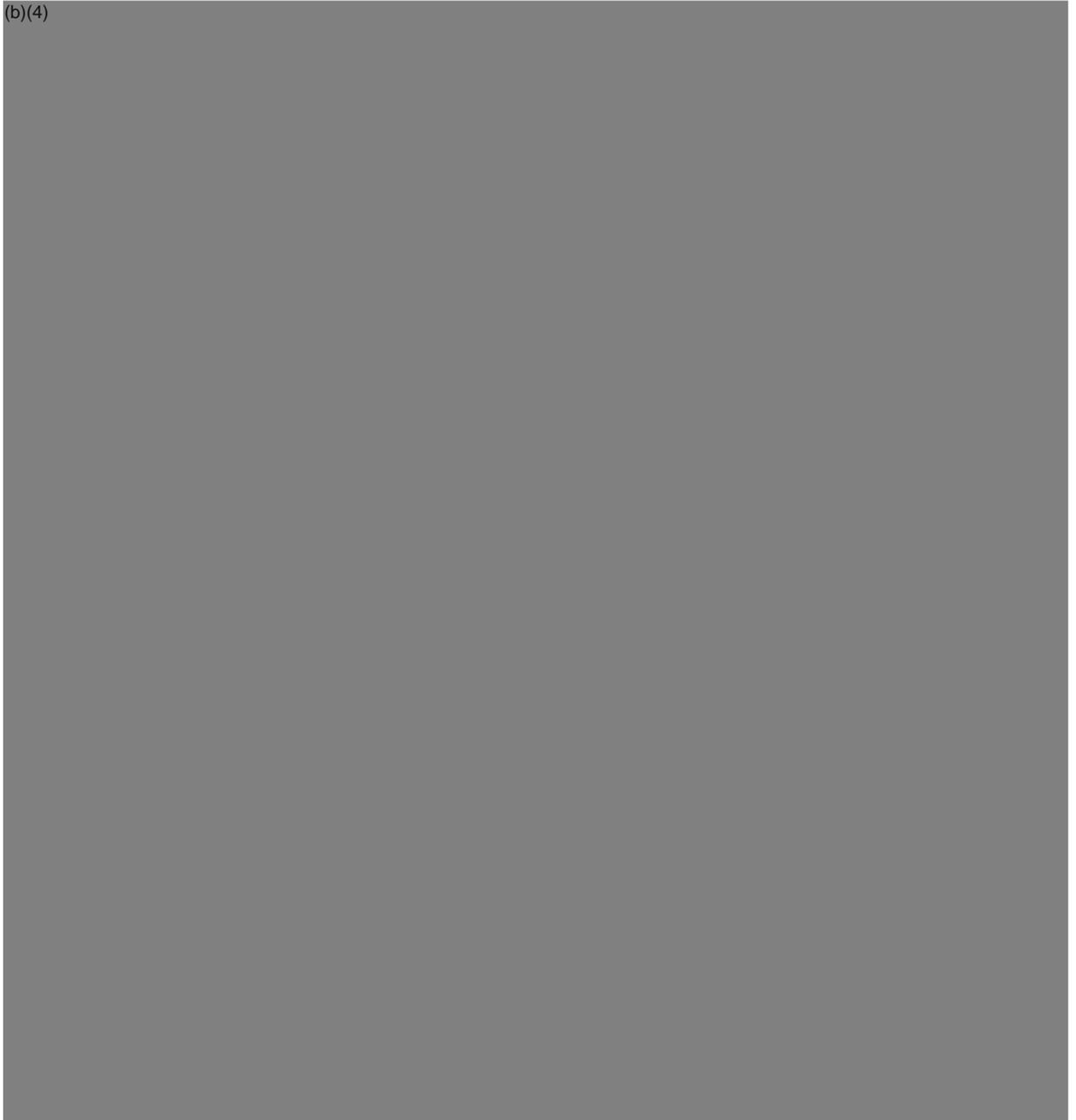
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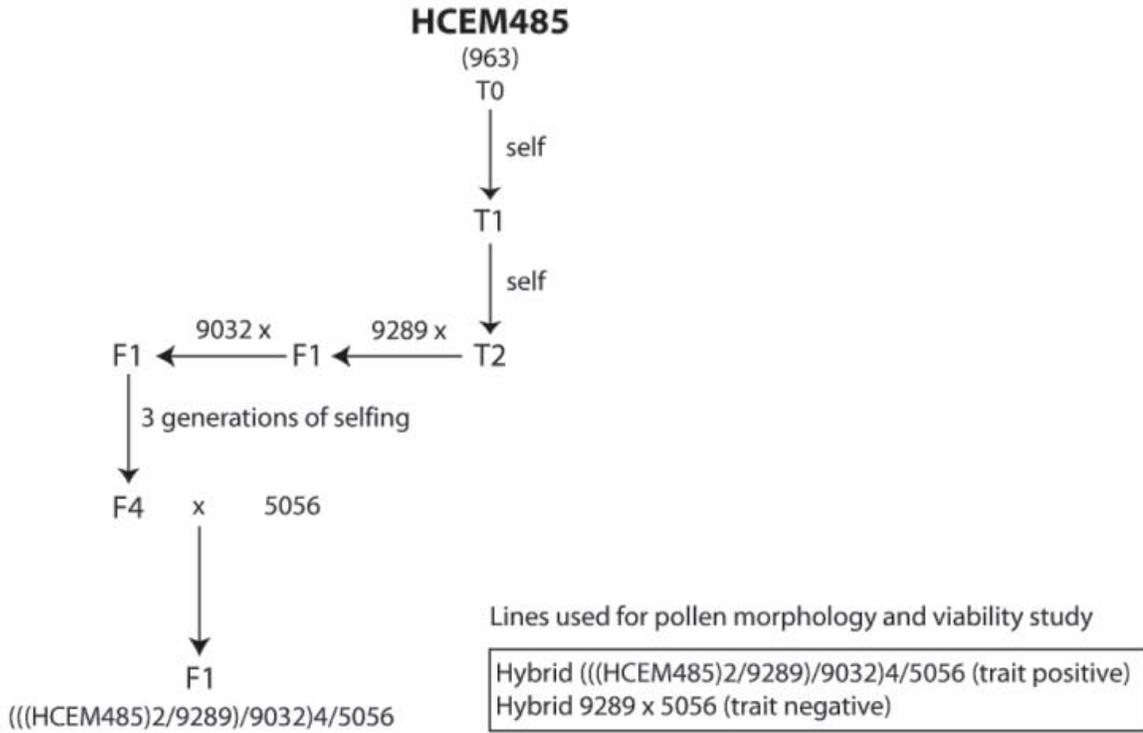
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1. INTRODUCTION

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REPORT NUMBER

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REPORT COMPLETED ON

25 July 2008

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STUDY SPONSOR

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

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Summary

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REPRESENTATIVE OF THE SPONSOR:

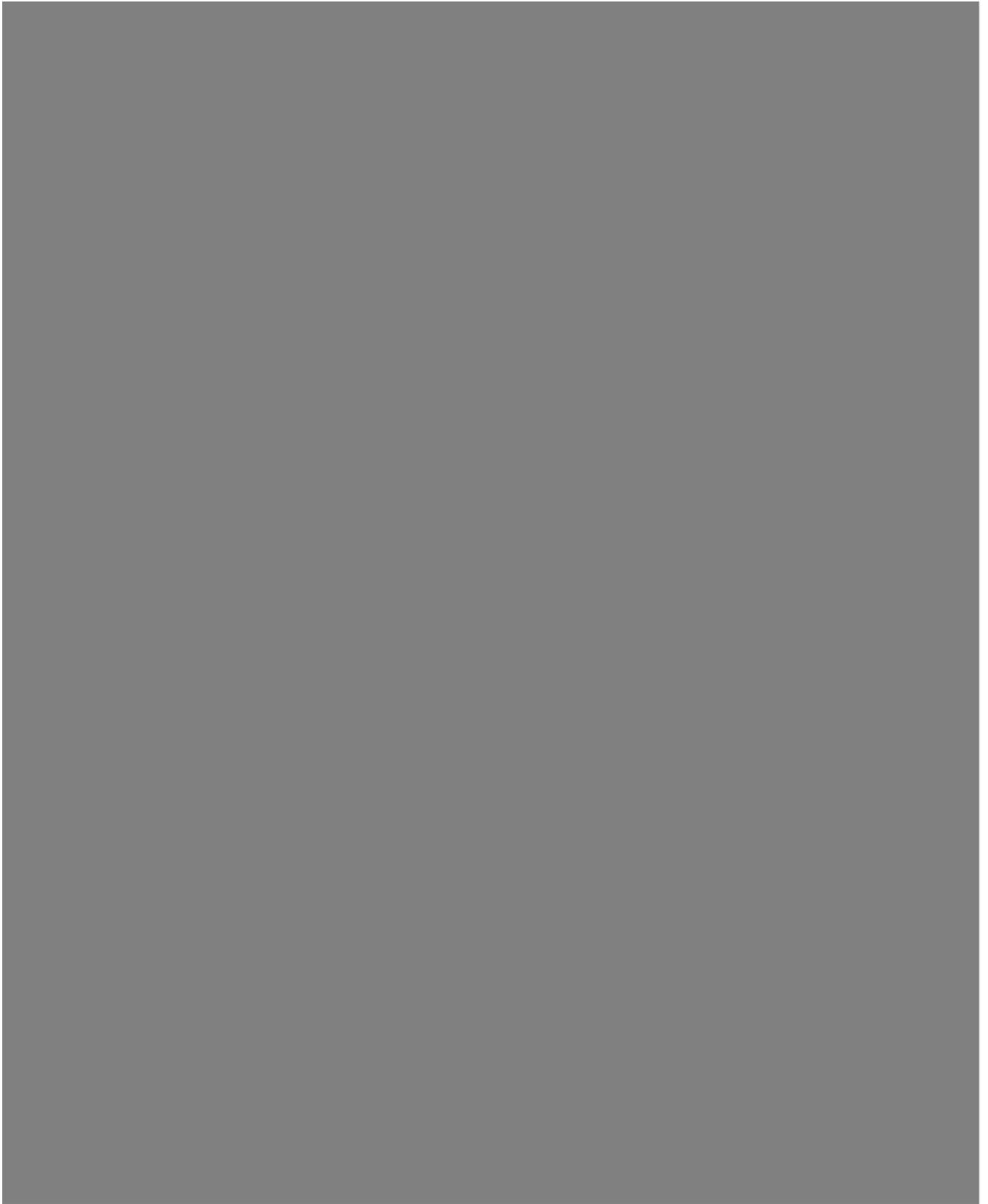
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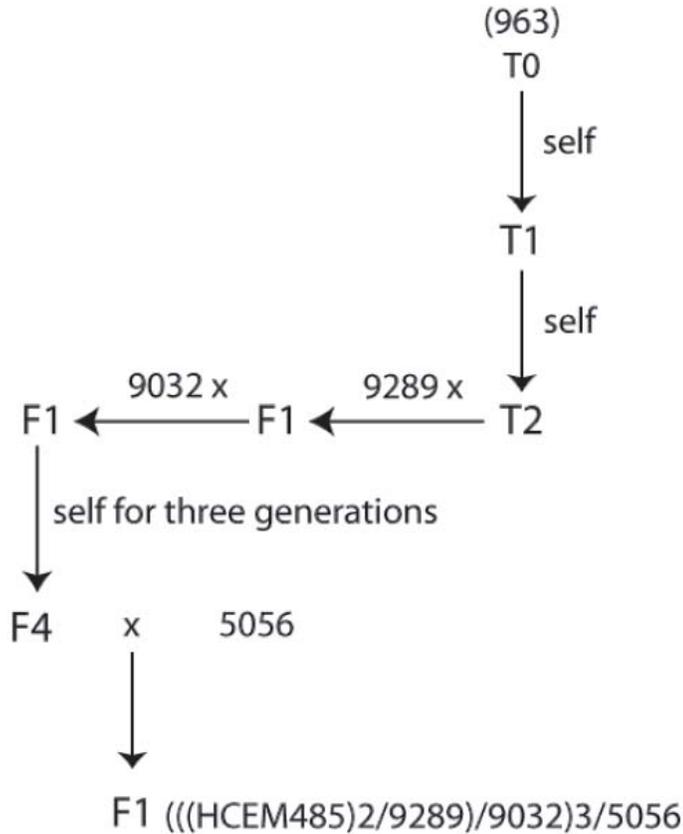
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Event HCEM485



<p>2007 Field Trials HCEM485 Hybrid (((HCEM485)2/9289)/9032)3/5056 (trait positive) Control Hybrid 9289/5056 (trait negative) Control Hybrid 9032/5056 (trait negative) Control Hybrid 963/5056 (trait negative)</p>

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USDA Extension Petition**

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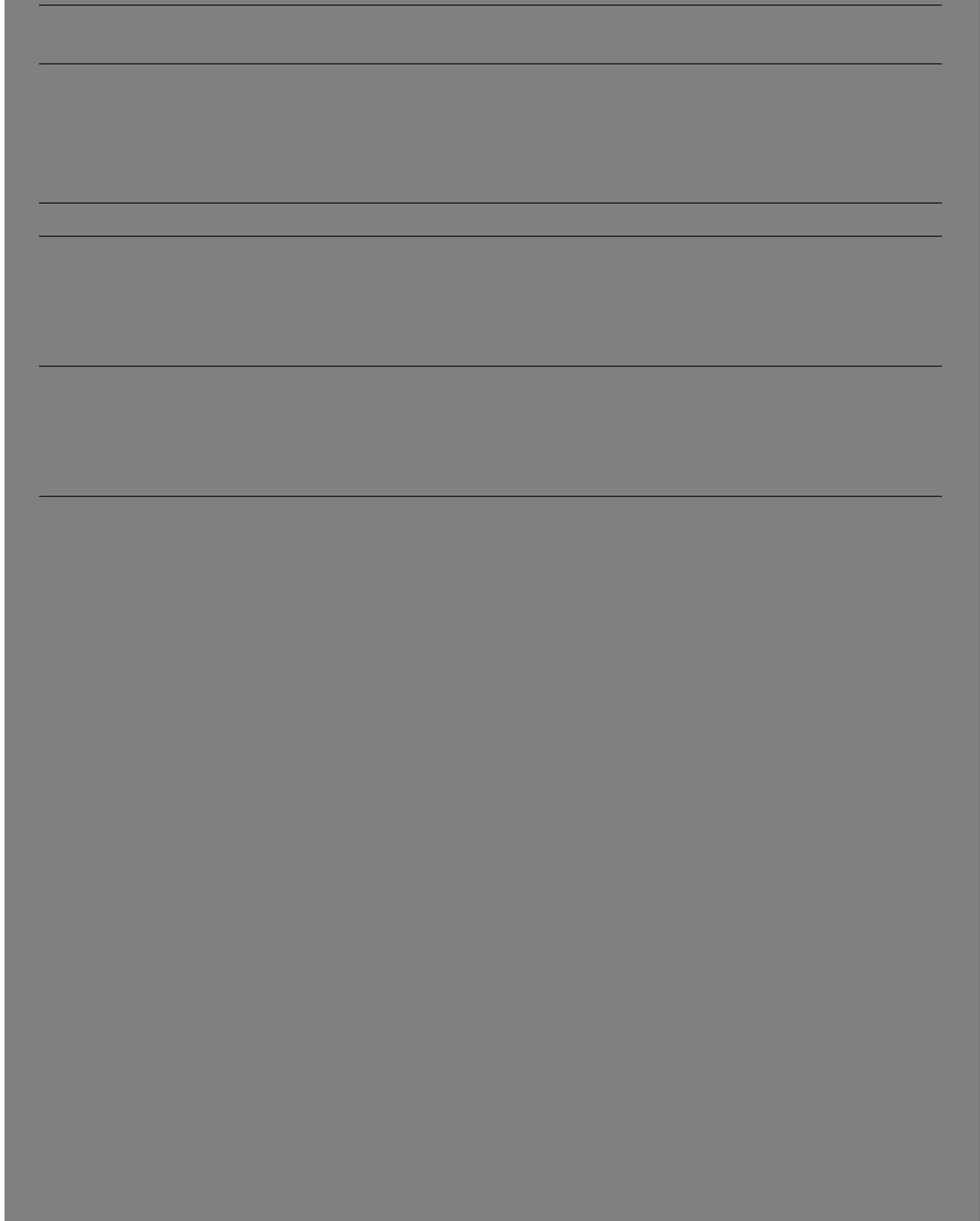
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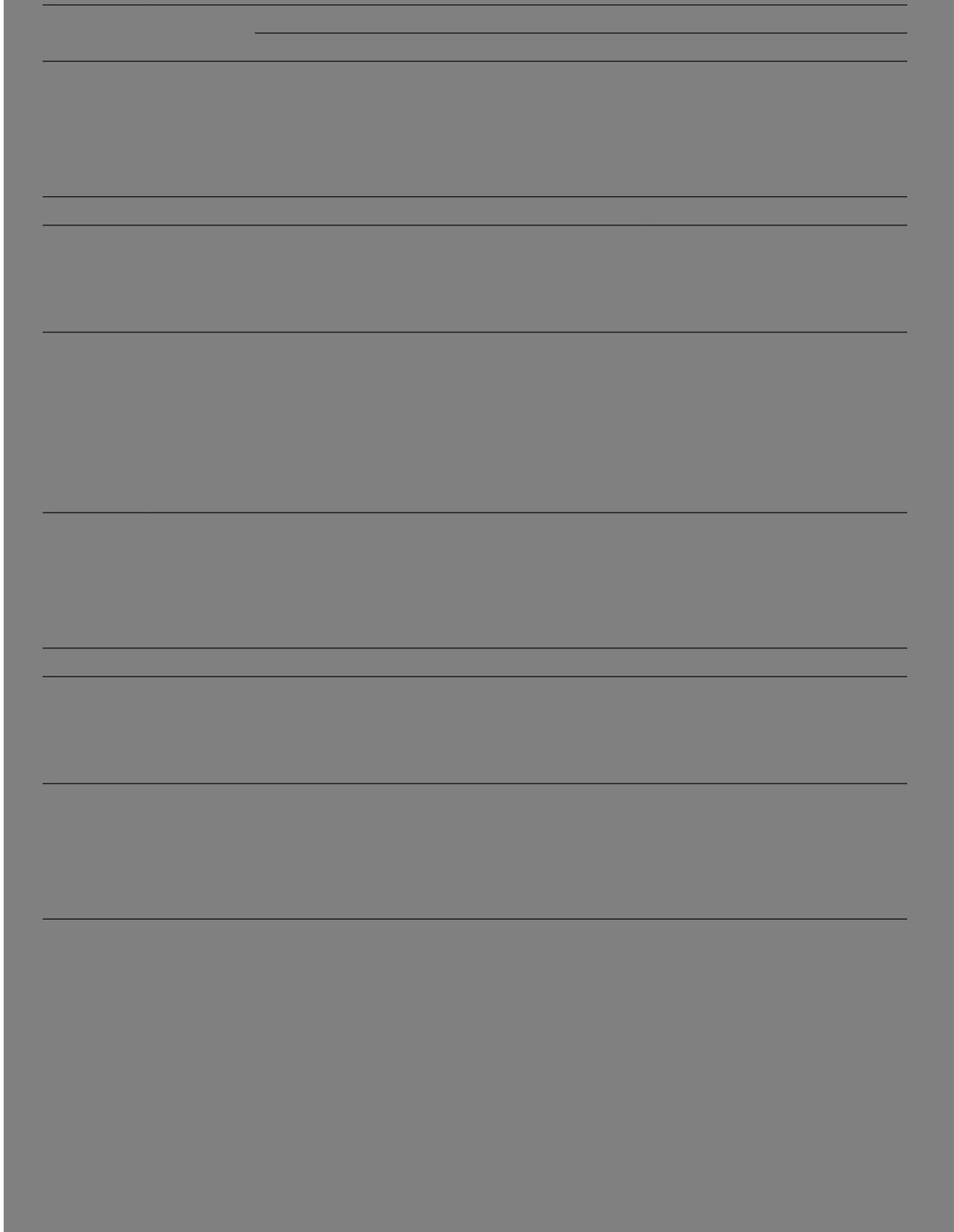
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Approved by

Feb. 24, 2009
Date

6. RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Stine Seed Farm, Inc., 22555 Laredo Trail, Adel, Iowa 50003.

7. REFERENCES

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