USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report (approx. May 08 – April 09)
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Cover Page

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Fiscal Year:	2008
USDA-ARS Agreement ID:	59-0790-6-064
USDA-ARS Agreement Title:	Genetic Diversity in and Genetic Mapping of Gibberella zeae.
FY08 USDA-ARS Award Amount:	$ 33,907

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
PBG	Genetic Diversity in and Genetic Mapping of Gibberella zeae.	$33,907
	Total Award Amount	**$ 33,907**

15 July 2009
Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
BAR-CP – Barley Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Winter Wheat Region
SWW – Southern Sinter Wheat Region

Project 1: *Genetic Diversity in and Genetic Mapping of Gibberella zeae.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

A number of lineages/phylogenetic species in *G. zeae* have been distinguished based in the genealogical concordance. There is considerable genetic diversity in gene sequence does exist between the lineages, but the lineages are not reproductively isolated under laboratory conditions suggesting that the lineages are not distinct species under the biological species concept. If these lineages are accepted as species, then trade restrictions and quarantine issues will certainly follow.

We have been characterized *G. zeae* populations from North and South America, Mexico and Korea for diversity for variation within and between lineages. There may be differences between lineages 6 and 7 in terms of the substrate required for completion of the sexual cycle, and toxin production varies at least generally with lineage. Naturally occurring hybrids are known, but not numerous, and can be found only outside the United States as one lineage dominates within the USA. Our goal is to evaluate the integrity of the proposed lineages and to test the hypothesis that these lineages are genetically isolated from one another DNA sequences similar to those of *ppg2* gene encodes a fungal sex pheromone in many heterothallic ascomycete fungi (requires two strains to mate and form the sexual stage). As *G. zeae* is homothallic, individual strains need not have a partner to complete the sexual portion of the life cycle. Heterothallic fungi have two sex pheromones (one for each of the two mating types) and use both to ensure that proper matings occur. The *ppg1* and *ppg2* genes encode these pheromones and the sequences are both found in *G. zeae*; however, only *ppg1* is functional and *ppg2* is likely to be a nonselected and non functional pseudogene. Such pseudogenes differ fundamentally from coding genes in that their entire DNA sequence can be changed without altering function, while most genes used for phylogenetic analyses are limited in their variation due to coding constraints required for the resulting protein to remain functional.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

Accomplishment:

We sequenced the entire *ppg2* gene (promoter, protein coding sequence, and terminator) from >100 strains from the nine of the described lineages /phylogenetic species. The relationships amongst these sequences is complex and cannot be interpreted as corresponding to the existing lineages. In the promoter region the following lineages and species (FP = *F. pseudograminearum*, FCu = *F. culmorum*, FCr = *F. crookwellense*) share identical sequences: (i) L2 & L3, (ii) L3 & L8, (iii) L5 & L9, and (iv) L1, L7 & L8, and there are unique sequences in FCr, FCu, L3, L4, L6, L7, and L8.

In the protein coding region there are two levels of variation – one involving a series of duplications and the other involving single nucleotide polymorphisms (SNPs) within the different duplicated regions. There are four basic formats for the coding region, none of these

formats includes an active prenylation site at the end of the polypeptide that is thought to be necessary for PPG2 function. The first form (L1 & L8) encodes just the 21 amino acids associated with the pheromone in other fungi. The second form (L2, L3, L6, L7 & L8) has the 21 amino acid sequence fused to a 19 amino acid sequence (40 amino acids total) that is virtually identical to the original unit but without the first two amino acids. The third form (L3 & L8)has the 21 amino acid sequence fused to a 16 amino acid sequence (37 amino acids total) that is virtually identical to the original unit without the first five amino acids. The fourth form (L3, L4, L5, L9, FP, FCu & FCr) has the 21 amino acid sequence fused sequentially to the 19 amino acid sequence and then to the 16 amino acid sequence (56 amino acids total). The last three *ppg2* forms are unique to *G. zeae* and are not known from any other completely sequenced fungus. Within the coding regions there are SNPs that generate common sequences for: (i) L1 & L7, (ii) L5 & L9, (iii) L2, L3 & L7, (iv) L3, L6 & L8, L4 &FCu, and (v) L4 & FP, and unique sequences for FCr, L3, L4, L5, and L8.

The terminator region also has two levels of complexity. There are two large insertions/deletions (indels). The terminator associated with coding region forms 1 and 3 has one of the indels, coding region form 2 has just the other indel, and coding form 4 has both indels. Again there are numerous SNPs within the terminator region that generate common sequences for: (i) L2 & L3, (ii) L4 & FP, (iii) L5 & L9, (iv) L1 & L7, and (v) L3, L7 & L8, and unique sequences for L3, L4, L6, L7, L8, FCr, and FCu.

Impact:

The taxonomic status of *G. zeae*/*F. graminearum* is of critical importance for plant quarantine and trade measures. If there are a number of species then each must be treated separately and the presence/absence of a particular species can be used as a non-tariff trade barrier. Our results suggest that while isolated populations of *F. graminearum* may exist, these populations are not reproductively isolated and should be recognized as portions of a single, large, diverse species rather than as multiple discrete entities. Such recognition would not materially impact the plant quarantine regulations currently in place, nor alter the application/implementation of current trade practices.

The question of naming different strains of *F. graminearum* is controversial. Previously this controversy has been interpreted as a difference in species definition, *i.e.* morphology or cross-fertility vs. phylogenetics. Using morphology and cross-fertility as measures, the nine lineages are not resolvable. DNA markers (AFLPs) and DNA sequences can group the isolates, but the members of these groups are cross-fertile under laboratory conditions. There are two critical questions: (1) How much sequence variation is present in the United States? and whether this variation can discriminate between the two different DON-associated genotypes? (2) Are there traits of economic importance within the various lineages and how frequently are these traits transferred between lineages. The *ppg2* gene is the first gene to be analyzed outside the *TRI* gene cluster that clearly does not follow the phylogenetic structure of the currently established lineages/phylogenetic species.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

1. Bentley, A. R., **J. F. Leslie**, E. C. Y. Liew, L. W. Burgess & B. A. Summerell. 2008. Genetic structure of *Fusarium pseudograminearum* populations from the Australian grain belt. *Phytopathology* **98:** 250-255.
2. Bowden, R. L., I. Fuentes-Bueno, **J. F. Leslie**, J. Lee & Y.-W. Lee. 2008. Methods for detecting chromosomal rearrangements in *Gibberella zeae*. *Cereal Research Communications* **36 (suppl. B):** 603-608.
3. Lee, J., **J. F. Leslie** & R. L. Bowden. 2008. Expression and function of sex phermones and receptors in the homothallic ascomycete *Gibberella zeae*. *Eukaryotic Cell* **7:** 1211-1221.
4. Lee, J., J. E. Jurgenson, **J. F. Leslie** & R. L. Bowden. 2008. Alignment of genetic and physical maps of *Gibberella zeae*. *Applied and Environmental Microbiology* **74:** 2349-2359.
5. **Leslie, J. F.**, and R. L. Bowden. 2008. *Fusarium graminearum*: When species concepts collide. *Cereal Research Communications* **36 (suppl. B):** 609-615.
6. Bandyopadhyay, R., R. A. Frederiksen & **J. F. Leslie**. 2008. Priorities for mycotoxin research in Africa identified by using the nominal group technique. In: *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade* (J. F. Leslie, R. Bandyopadhyay & A. Visconti, eds.), pp. 19-26. CABI, Kew, UK.
7. Coulibaly, O., K. Hell, R. Bandyopadhyay, S. Hounkponou & **J. F. Leslie**. 2008. Economic impact of aflatoxin contamination in Sub-Saharan Africa. In: *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade* (J. F. Leslie, R. Bandyopadhyay & A. Visconti, eds.), pp. 67-76. CABI, Kew, UK.
8. Shelton, B. G. & **J. F. Leslie.** 2008. Comparative risks of airborne and foodborne molds and mycotoxins. In: *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade* (J. F. Leslie, R. Bandyopadhyay & A. Visconti, eds.), pp. 317-324. CABI, Kew, UK.
9. Lee, J., **J. F. Leslie** & R. L. Bowden. 2008. Functions of the sex pheromones in *Gibberella zeae*. *Rivista di Patologia Vegetale* **90:** S3.26.
10. **Leslie, J. F.**, J. Lee, J. E. Jurgenson & R. L. Bowden. 2008. An update of the genetic map of *Gibberella zeae*. *Rivista di Patologia Vegetale* **90:** S3.26.
11. Lima, C. S., S. S. Costa, M. A. Campos, **J. F. Leslie** & L. H. Pfenning. 2008. Etiology of mango malformation and PCR detection of its causal agent in Brazil. *Rivista di Patologia Vegetale* **90:** S3.65.
12. Minnaar-Ontong, A., L. Herselman, W. M. Kriel & **J. F. Leslie**. 2008. Population dynamics of Fusarium Head Blight in South Africa. *Rivista di Patologia Vegetale* **90:** S3.66.

Research presentations made at:
1. Norwegian National Veterinary Institute, Oslo, Norway – 04/08.
2. Faculty of Agricultural & Life Sciences, Seoul National University, Seoul, Korea – 05/08.
3. Science University of Malaysia, Penang, Malaysia – 06/08.
4. IUMS International Mycology Congress, Istanbul, Turkey – 08/08.
5. International Wheat Scab Symposium, Szeged, Hungary – 09/08.

6. Pan-African Environmental Mutagenesis Conference, Cape Town, South Africa – 11/08.
7. Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa – 11/08.

If your FY08 USDA-ARS Grant contained a VDHR-related project, include below a list all germplasm or cultivars released with full or partial support of the USWBSI. List the release notice or publication. Briefly describe the level of FHB resistance. If this is not applicable (i.e. no VDHR-related project) to your FY08 grant, please insert 'Not Applicable' below.

Not Applicable