Differences in midgut gene expression between Bt exposed and unexposed Western bean cutworm

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Abstract
The control of the Western bean cutworm (WBC) by transgenic corn hybrids that express *Bacillus thuringiensis* (Bt) has diminished over the past decade, such that crop damage is routinely observed in some regions of the United States and Canada. The midgut cellular response of WBC Cry1F resistant larvae was investigated at the transcriptional level by comparison of RNA sequencing (RNA-seq) data between full-sibs either exposed or unexposed to a sub-lethal Cry1F dose via toxin overlay bioassay (10,000 ng cm^{-2}). Out of 52,371 assembled transcripts, 542 and 1,393 were respectively up- and down-regulated (Log2 fold change ≥ 2.0 and FDR ≤ 0.05). Among these transcripts, the most highly up- and down-regulated genes respectively encode a glycoside hydrolase and a nuclear hormone receptor-like protein. No differential expression was predicted for transcripts from previously identified candidate Bt resistance genes (e.g. ABC transporters, aminopeptidase N, alkaline phosphatase, or cadherin). This information is important for understanding any variance in cellular responses to Cry1Fa toxin among resistant WBC larvae.

Methods
WBC eggs were collected from Herculex (Cry1Fa toxin expressing) transgenic maize at the University of Nebraska West Central Water Resources Field Laboratory near Brule, NE in July 2015, and transported to the USDA-ARS Corn Insects & Crop Genetics Research Unit, Ames, IA. Each newly hatched larvae was used to infest a single cell of a 128-cell bioassay tray containing artificial diet over laid with Cry1Fa toxin at either 10,000 or 5,000 ng cm^{-2} (**Table 1**) as described previously [1]. Larvae were weighed after 11 days and transferred to bioassay tray cells containing diet wither either 5,000 or 0 ng cm^{-2} Cry1Fa toxin, and reared for an additional 5 days. Midgut tissues were then dissected larvae from egg mass B1, total RNA extracted using the DirectZol RNA Miniprep Kit (Zymo Research), and samples submitted to the Iowa State University DNA Facility paired end 100 bp reads generation on an Illumina HiSeq2500. Raw reads were trimmed and then assembled into a combined reference transcriptome using Trinity [2], to which each set of trimmed reads were mapped using Bowtie2 [3] and significance of differences in read counts (expression) assessed with DESeq2 (citation). Differentially-expressed transcripts were annotated using Blast2GO [4].

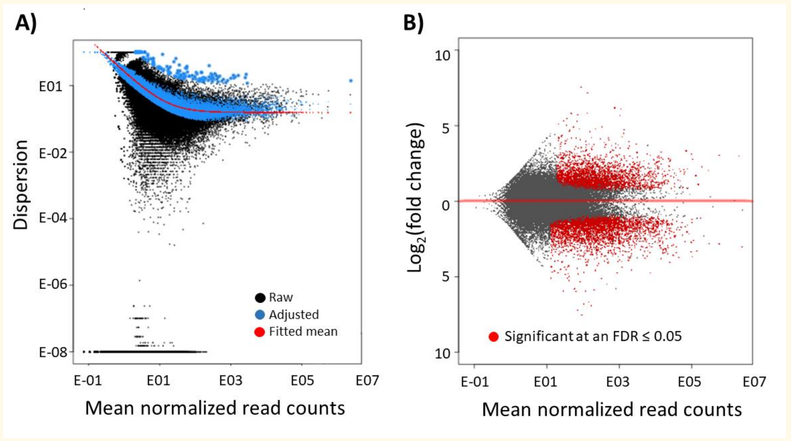
Table 1: WBC samples for RNA-seq

		Bioassay: day 1-11		Diet: day 12-16
Eggmass	Individual	Cry1Fa*	Wt (mg)	Cry1Fa*
B1	1	10,000	0.0329	5,000
B1	2	10,000	0.0291	5,000
B1	3	10,000	0.0368	5,000
B1	4	10,000	0.0390	5,000
B1	12	10,000	0.0275	5,000
B1	21	5,000	0.0254	5,000
B1	22	5,000	0.0368	5,000
B1	23	5,000	0.0250	5,000
B1	34	5,000	0.0117	0
B1	35	5,000	0.0085	0
B1	36	5,000	0.0107	0
B1	37	5,000	0.0041	0
B1	38	5,000	0.0064	0

* Units as ng cm^{-2}

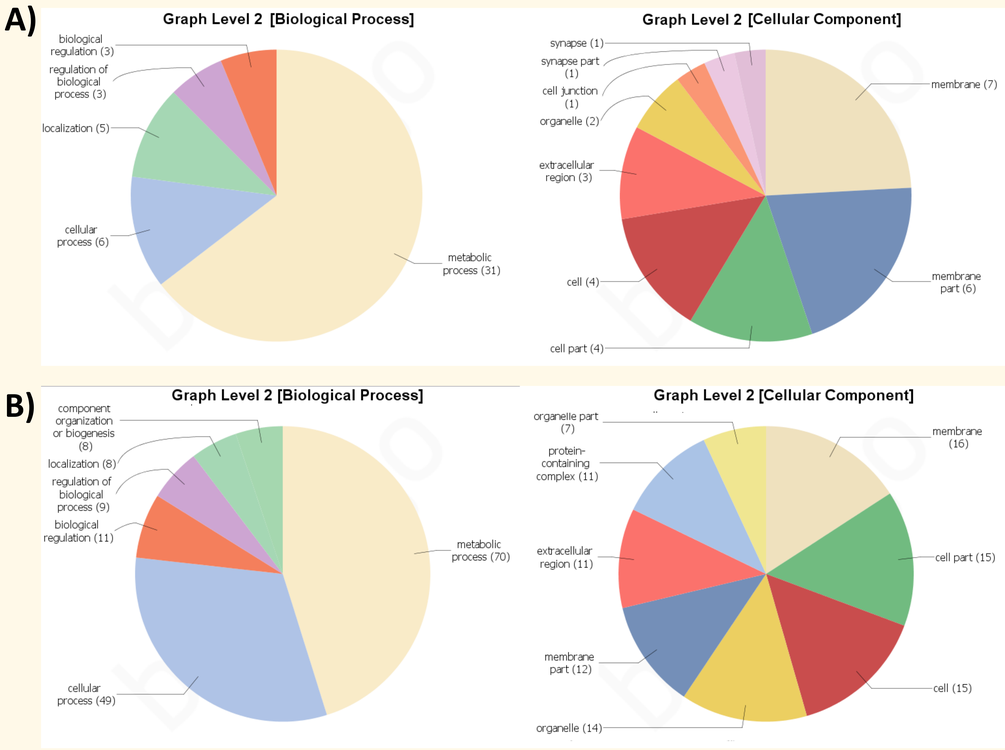
Results
The Trinity assembler generated 52,371 transcripts of which 38,725 were predicted to encode protein ≥ 100 amino acids. Mapping of individual RNA-seq reads to these transcripts and normalization reads by DESeq2 (**Fig 1A**), resulted in 542 up- and 1,393 significantly down-regulated transcripts (FDR ≤ 0.05; **Fig 1B**) for the comparison between larvae fed artificial diet with or without Cry1Fa toxin (**Table 1**).

Figure 1: RNA-seq normalization (A) and significance (B)



Compare to up-regulated genes, the functional annotations for cell component show an enrichment of organelle and protein complexes among down-regulated genes in Cry1Fa exposed larvae (**Fig. 2**), greater proportion involved in cell component organization or biogenesis.

Figure 2: Gene ontology annotations for transcripts **A)** up-regulated **B)** down-regulated in Cry1Fa toxin exposed WBC larvae.



Discussion and Conclusions
WBC has developed high levels of resistance to Cry1Fa toxin expressed by transgenic corn, and a greater understanding of WBC adaptation that allow this feeding to occur may be informative for developing novel methods to circumvent crop damage. The WBC transcriptome assembled here represent the first for the midgut of this species. DESeq2 analysis of RNA-seq data between more resistant WBC larvae fed 5 days on Cry1Fa toxin compared to less resistant larvae from the same egg mass (e.g. full- or half-sibs) fed 5 days on control (non-Cry1Fa) diet provided insight into the possible responses of resistant genotypes to Cry1Fa toxin. Putative gene functions suggest major change might occur to the structural composition of organelles during Cry1Fa exposure.

References
Available from corresponding author upon request