Research Proposal for the

Graduate Student Research and Professional Development Fund

The School of Graduate Studies

Western Illinois University

1 University Circle Macomb, IL 61455

Submitted by:

XXXXXX

Adviser: Sue Hum-Musser, Ph.D.

Department of Biological Sciences

Waggoner Hall 372

1 University Circle Macomb, IL 61455

Date of Submission: September 15, 2009

Title of Research: Ozone Weakens Plant Defenses against Caterpillar Insect Pests

Background

Ozone is often considered a double-edged sword. In the upper atmosphere it acts as a protective shield against the harmful ultraviolet rays from the sun; however in the lower atmosphere it is one of the hazardous air pollutants. Ozone has been known to impair lung functions and reduce agricultural productions (NASA, 2010).

The destructive effect of ozone on plant defenses stems from its ability to improperly turn on the salicylic acid- (SA) mediated pathway which is widely known to be responsible for defenses against pathogens, such as bacteria and viruses (Sharma et al., 1996). The other well-known pathway is the jasmonic acid-(JA) mediated pathway which is involved in defenses against environmental stresses and herbivory. When one pathway is triggered, the other one is turned off because of cross-talk between these pathways. When the SA pathway is induced, the plant is unable to effectively defend itself against herbivores, such the generalist *Helicoverpa zea* caterpillar (Figure 1), infamously known as the corn earworm (Smith et al., 2009). The corn earworm pest causes significant economic losses from destruction of agricultural crops such as corn, tomato, cotton, and soybean (Steffey et al., 1998). In the presence of ozone pollution, plants turn on the SA pathway (Sharma et al., 1996); however insect herbivory induces the expression of the JA pathway (Smith et al., 2009). I will test the combined effect of ozone and caterpillar feeding damage tomato plants. If ozone treatment on plants results in ineffective defense against insect pests, this will result in the increase of pesticide use, which is harmful to the environment.

Figure 1. *Helicoverpa zea* larva (Courtesy of Benson Research).

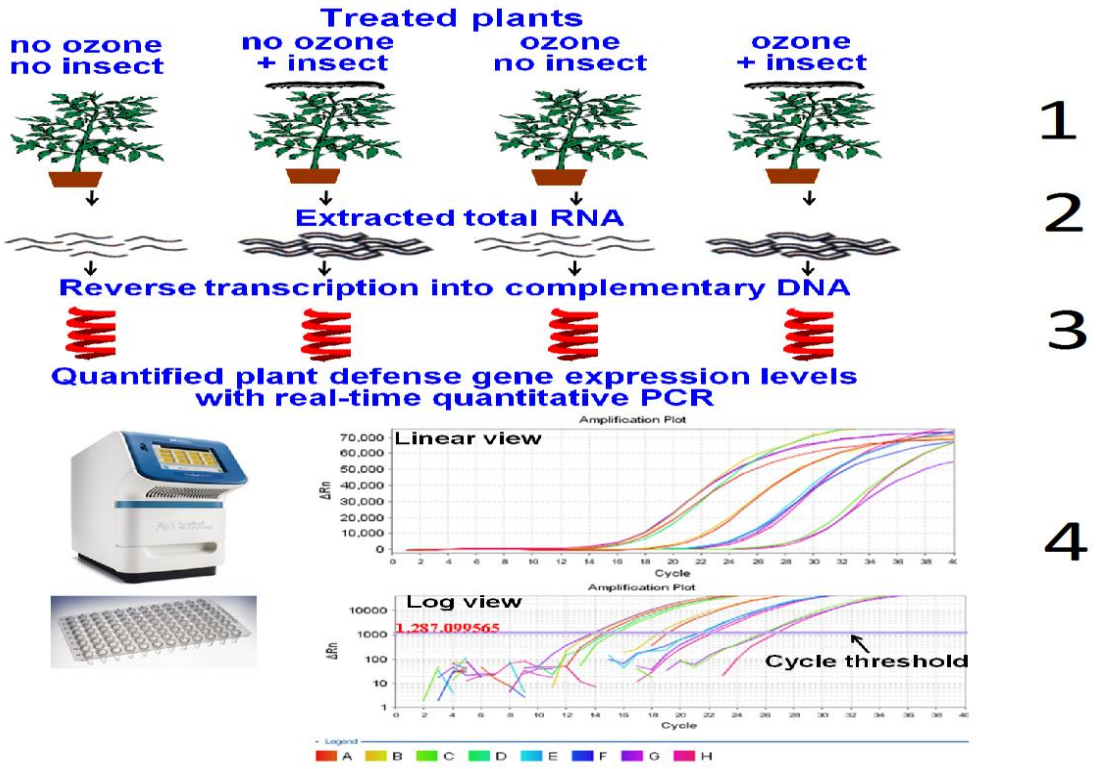
The objective of this study is to determine the gene expression of tomato plants when challenged by ozone and herbivory from insect caterpillars separately and simultaneously. Plant defense responses to

both these stresses have not been explored in detail at the gene expression level. We will be using the insect pest, *H. zea* and tomato plants, *Solanum lycopersicum* cv 'Beefsteak'. The United States is the world's second largest tomato producer behind The People's Republic of China. Tomato products account for more than US$2 billion in annual farm cash receipts (ERS-USDA, 2010). The increasing level of ozone in the lower atmosphere, resulting from both natural and human causes, could be devastating on tomato production in the future. This research may result in potential development of new methods of increased plant performance and yield.

Methodology

Sixteen tomato plants will be divided into 4 groups (Figure 2). Each group will receive one treatment. The control group will receive no treatment. The second group will be exposed to feeding from 12 *H. zea* caterpillars. The third group will receive 15 parts per million of ozone treatment. The last group will be subjected to both ozone and feeding from 12 *H. zea* caterpillars. The caterpillars will be allowed to feed on the leaves for 24 hours. We will harvest plant leaves and freeze them in liquid nitrogen to prevent tissue degradation. (Figure 2, Step 1).

Figure 2. Flow chart of the methodology



All organisms produce messenger RNA (mRNA) from a variety of genes in response to stimuli from the environment. The products of gene expression determine how the plant reacts to those stimuli. We can determine the plant pathways affected by the treatments by quantifying the mRNA levels of different genes in the plant defense pathways. The mRNA will be extracted from the tissue (Step 2). We will make complementary DNA through reverse transcription (Step 3). Using the complementary DNA, we can measure the different levels of gene expression in the plants with a process called real-time quantitative polymerase chain reaction (qPCR) (Step 4). We can analyze the qPCR data and determine which plant defense pathways are turned on or off based on the gene expression levels. Figure 3 shows preliminary data that demonstrate the expression of important genes in the JA-mediated pathway. All these genes are turned on in response to caterpillar feeding in air, but when plants are exposed to ozone and caterpillar herbivory, each of these gene expression levels is reduced. These data support our

hypothesis that in ozone polluted environments, plants are not as effective in responding to insect damage. I will run eight replicates of each treatment to obtain reliable and reproducible data.

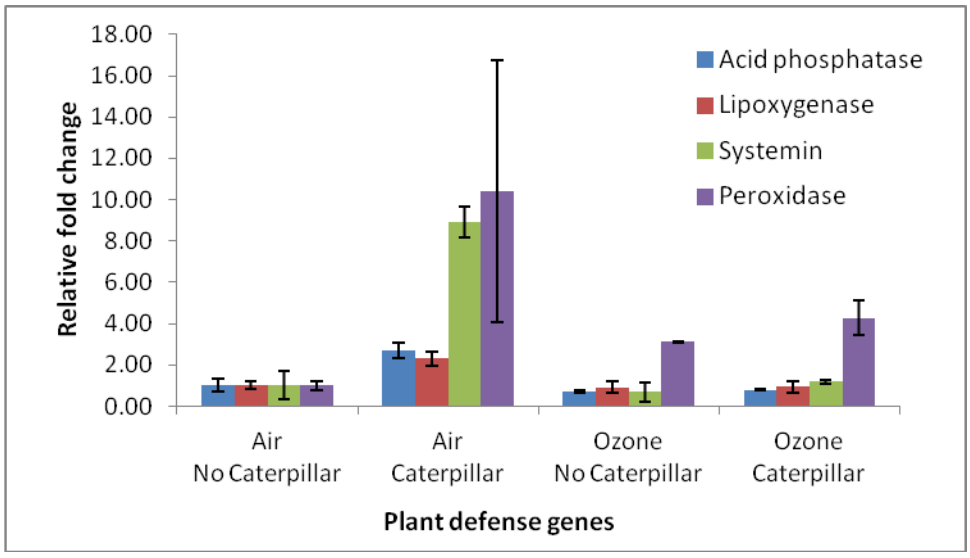


Figure 3. Gene expression levels of different plant defense genes when tomato plants are exposed to feeding damage by insect pests and ozone pollution separately and combined.

I intend to examine the expression of other genes in both the SA- and JA-mediated plant defenses by running more qPCR assays, and enzyme assays. I also plan to determine the growth and survivability of newly hatched *H. zea* caterpillars (neonates) of plants treated with ozone. Each neonate caterpillar will be placed on tomato leaves pre-treated with ozone or air. I will use about 50 caterpillars as replicates in each treatment to obtain reliable data. I will record caterpillar survival daily and caterpillar weight every 3-4 days until they turn into pupae. Dates of moth emergence from the pupa state will also be recorded. This data will provide information on how the insects are affected by feeding on plant defense compounds as the plants respond to ozone or air treatments. I anticipate that caterpillars feeding on ozone-treated plants will grow faster, pupate, and emerge sooner than caterpillars feeding on air-treated plants. This expectation is due to the decrease in plant defenses specific against insects, because ozone has increased expression of the SA-pathway and reduced induction of the JA-pathway (against insects).

References

Benzon Research - Insect Research and Sales. Website accessed on 12 Sept. 2010. <http://www.benzonresearch.com/>.

NASA Earth Observatory. Watching Our Ozone Weather: Feature Articles. Website accessed 12 Sept. 2010. <http://earthobservatory.nasa.gov/Features/OzoneWx/>.

Sharma YK, Raskin I, Davis KR. 1996. Ozone-induced Responses in Arabidopsis Thaliana: the Role of Salicylic Acid in the Accumulation of Defense-related Transcripts and Induced Resistance. Proc Natl Acad Sci U S *A.* 93.10: 5099-104.

Smith JL, De Moraes CM, Mescher MC. 2009. Jasmonate- and Salycylate-mediated Plant Defense Responses to Insect Hebivores, Pathogens and Parasitic Plants. Pest Manag Sci 65: 497-503.

Steffey K, Rice M, All J, Andow J, Van Duyn J, Gray M Editors. 1999. Handbook of Corn Insect Pests. Entomological Society of America, Landham, Maryland.

USDA Economic Research Service. ERS/USDA Briefing Room - Vegetables and Melons: Tomatoes. Website accessed 12 Sept. 2010. <http://www.ers.usda.gov/Briefing/Vegetables/tomatoes.htm>.

Budget

This research is partially supported by grants through my advisors, Drs. Hum-Musser and Musser. Caterpillars will be obtained through a collaboration with the USDA-ARS office in Peoria, except for the cost of shipping. The cost running molecular techniques is very expensive. It approximately costs $90 to run a qPCR plate and we can only analyze 5 genes per plate. We would like to examine more genes and acquiring this grant would be a big help.

Item Description	Grad Dev Fund	CAS Match Fund	Drs. Hum-Musser and Musser's grant Funds	Total $
H. zea caterpillar shipping	-	-	$76	$76
Plant seeds, soil, pots			$50	$50
Trizol reagent for RNA extraction	-	$250	$10	$260
Other reagents for RNA	-	-	$250	$250

extraction, plasticware				
Liquid nitrogen for freezing tissue and RNA extraction	-	-	$152	$152
Reverse transcription kit for making cDNA	-	-	$242	$242
qPCR reagents	$500	-	$560	$1060
Enzyme reagents	-	-	$250	$250
Total costs	**$500**	**$250**	**$1590**	**$2340**

I will also be presenting a poster on this research at the national meeting of Entomological Society of America this December. Funds to offset the cost of attending this meeting will be partially paid for by grants through my advisors and personal funds.