Controlling *Lygus lineolaris* pest population

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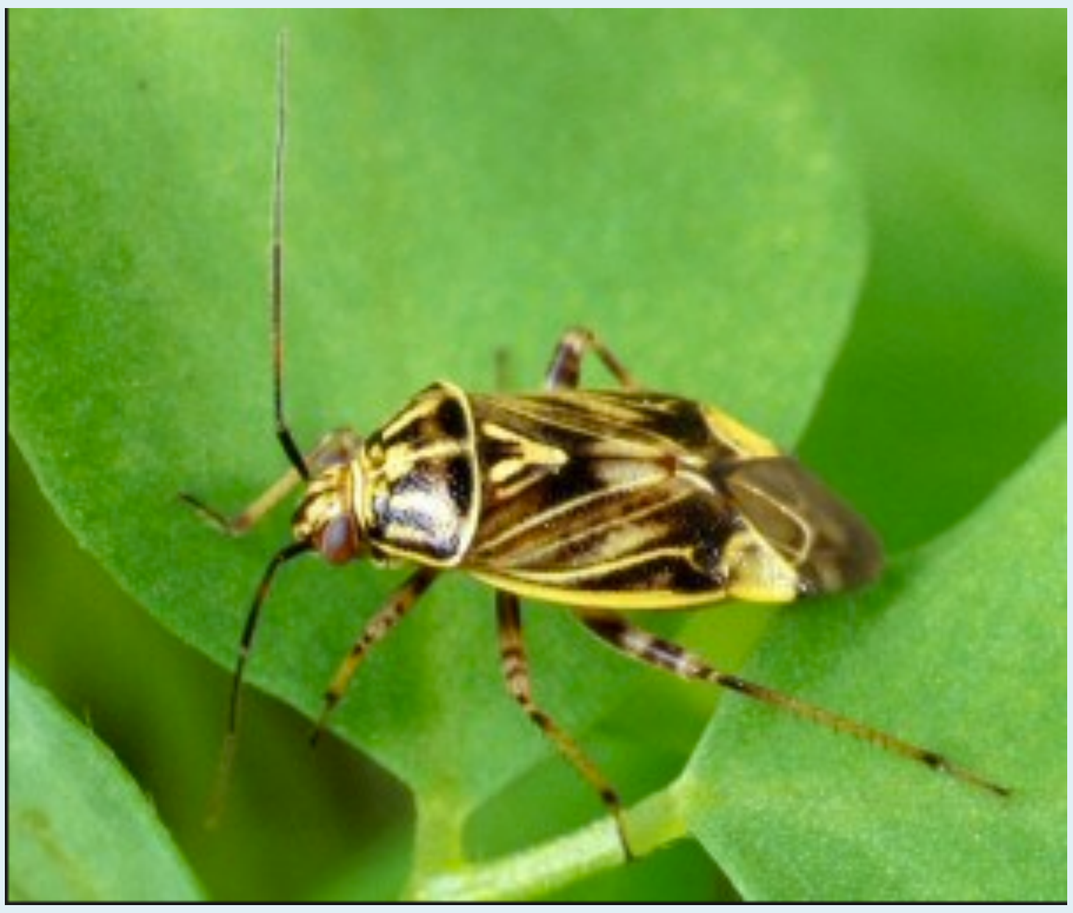
INTRODUCTION:
Lygus lineolaris is a common pest affecting agricultural crops in North America. Chemical treatments to manage this pest have not been effective, and there is a need to develop molecular based strategies based on the insect's biology, physiology, genetics and ecology. Antennae are the primary organ of olfaction used by Lygus to perceive and interact with its environment. This sensory organ allows the insect to detect and differentiate odors for the purpose of foraging and reproduction. This organ also houses the olfactory co-receptor (Orco). The interaction between Orco and an odorant specific receptor results in the formation of an ion channel in response to odorant binding. To understand the molecular basis of this system in the western tarnished plant bug, L. *hesperus* (LhOrco) was cloned and characterized. LhOrco has many functional properties and is expressed in antennae, proboscis, and legs. Hence, strategies that target disruption of this gene product at the transcript or protein level may facilitate an effective means for controlling Lygus pest populations.

MATERIALS AND METHODS:
Lygus lineolaris were obtained from USDA ARS Southern Insect Management Research Unit, Stoneville, MS. RNA was extracted from legs, antennae, and proboscis. Tissues from 10 insects were pooled in one extraction, and two extractions were performed for each tissue. Messenger RNA was purified from the total RNA using a Poly A(+) tract mRNA isolation system (Promega, Madison, WI). cDNA synthesis and labeled aRNA production was carried out using Agilent's aRNA production kit. Cy3 and Cy5 fluorescent dyes were used for labeling samples. Nanodrop instrument was used to obtain absorbance values for nucleic acids. The cDNA was then amplified using polymerase chain reaction. The amplified cDNA was run through an agarose gel and extracted and purified. The purified cDNA was then placed in TA cloning vectors to be used for sequencing. GeneSpring v9.3 software (Agilent) was used for data analyses. The bioinformatic analysis was done by BLASTIX to identify contig sequences related to other OBP sequences. Cloning and re-sequencing of OBPs were carried out with Invitrogen first-strand cDNA synthesis kit. Real time quantitative PCR analysis was done on total RNA extracted from various tissues harvested from 2-day old adult male and female *L. lineolaris*. Plasmid DNAs were sequence verified and linearized DNA was gel purified to determine DNA concentration.



RESULTS AND DISCUSSION:
Odorant binding proteins (OBP's) of insects are a family of 150 amino acid, water soluble protein characterized by six conserved Cys residues. Based on OBP classification and bioinformatics analyses, 33 OBP-like transcripts were identified to understand the biological functionality. Transcriptome approaches to identify the genes encoding proteins exhibiting OBP-like features conferred the molecular basis of olfaction in *L. lineolaris*. OBP-like transcripts in *L. lineolaris* were identified from multiple short sequence reads derived from a non-normalized whole body *L*. lineolaris. A few of the OBP's identified in transcriptome-based screen appear to be slice variants (molecular process that makes use of different exon splice sites in a gene to generate multiple transcripts encoding varying protein isoforms).

Olfaction plays a critical role in governing a number of essential behaviors to suggest targeted disruption of essential gene product either at the transcript or protein level to control Lygus pest populations.

REFERENCES:
Hull, J.J., O.P. Perera and G. L. Snodgrass. Transcriptome analysis and expression profile of odorant binding proteins in the tarnished plant bug *Lygus lineolaris*. Insect Mol Biol (submitted)

Chinta S, Dickens J, Aldrich J (1994). Olfactory reception of potential pheromones and plant odors by tarnished plant bug, *Lygus lineolaris (Hemiptera: Miridae). J Chem Ecol* ***20****: 3251-3267.*

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