Investigation of the Functions of Insect Arginine Vasopressin-like Peptides
Rogan Tokach\textsuperscript{1,2}, Fenwicht Edwardson\textsuperscript{1,2}, Donghun Kim\textsuperscript{1}, and Yoonseong Park\textsuperscript{1}
\textsuperscript{1}Department of Entomology, College of Agriculture, Kansas State University
\textsuperscript{2}Department of Animal Science, College of Agriculture, Kansas State University

\section*{Abstract}
The neuropeptide arginine vasopressin (AVP) is a multifunctional signaling peptide that is highly conserved across eukaryotic animals. In mammals, it primarily acts as an anti-diuretic and a regulator of blood pressure. These traits among others merit the study of the arginine vasopressin-like (AVPL) hormone found in insects (Aikins et al., 2008). We investigated the bioactivity of AVPL in an assay measuring the recovery time after knockout in water, indicating hormonal activity for alert states in the red flour beetle \textit{Tribolium castaneum}. We found that injections of an AVPL mimetic peptide (AVP1) prolonged the knockout alert states in the red flour beetle recovery time after knockout in water, indicating hormonal activity for survival.

\section*{Introduction}
\textit{Tribolium castaneum}, a notorious stored product pest, became a model genetic organism after the whole genome was sequenced. AVPL in this species of insect was previously identified, but the function is unknown yet. Suppression of the gene expression by using RNA interference (RNAi) of the AVPL gene found no significant developmental defects previously (Aikins et al., 2008). In this study, we used a new assay method that examined the activity of AVPL in the larval alert state by measuring recovery time after water submersion.

\section*{Question and Hypothesis}
\textbf{Question:} Whether AVPL mimetics have effects on the alert state and on the survival of \textit{T. castaneum}?
\textbf{Hypotheses:} Injections of AVPL mimetics into the larvae of \textit{T. castaneum} will change the recovery time from the knockout induced by water submersion. The injections will also affect the survivorship.

\section*{Methods and Experimental Design}
\begin{itemize}
\item Three injection solutions were prepared: Phosphate buffered saline (PBS), 10 \(\mu\)M AVP1, and 10 \(\mu\)M AVP2.
\item AVP1: A plant cyclotide that acts on the \textit{Tribolium} AVPL receptor as partial (Emax~50\%) biased agonist with an EC50 of ~5\(\mu\)M.
\item AVP2: An AVPL D-amino acid analogue which acts as full biased agonist with an EC50~90\(\mu\)M.
\item Sixth instar larvae were immobilized on double sticky tape and AVPL mimetics were injected with a 100 nL solution through abdominal segments (Figure 1A).
\item Following injection, larvae were submerged in water for 2 minutes (Figure 1B).
\item Larvae were then placed on a dry paper towel and monitored until they showed signs of movement. The recovery time was measured.
\item For measuring the long term survivorship, the insects were placed in individual wells of a 96-well plate with food and placed in an incubator (30°C, ~40% relative humidity).
\item Insect mortality was monitored for the 6 to 7 days following injection.
\end{itemize}

\section*{Results}
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\item Injections of AVP1 resulted in significant delay in the recovery time from the knockout induced by submersion in water (Figure 2).
\end{itemize}

\section*{Future Directions}
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\item We need to repeat the assays to confirm the results in recovery time assay and mortality assay.
\item We will include additional controls: uninjected control with and without immersion in water.
\item We would like to examine the effects of AVPL mimetics in other pest arthropods, including varroa mite.
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\section*{Acknowledgements}
We thank Dr. Christian Gruber for providing the peptides used. We also thank Dr. Jeremy Marshall for arranging the research opportunity.

\section*{References}
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