



Research Kernels

Our Latest Research Results – February 2015

Functional analysis of C1 family cysteine peptidases in the larval gut of *Tenebrio molitor* and *Tribolium castaneum*

Authors: A. Martynov, E. Elpindina, L. Perkin, B. Oppert
Submitted to: BMC Genomics

Insects digest proteins through a complex set of digestive enzymes. In some beetles, protein digestion relies on a set of enzymes called cysteine cathepsins. In tenebrionids, these cysteine cathepsins are in the first part of the digestive tract and are critical to digestion of their main food, cereal proteins. We have compared cysteine cathepsins in two tenebrionids, the red flour beetle and yellow mealworm, using high throughput sequencing. We find that each insect has approximately several dozen cysteine cathepsins, and it is apparent that many of these are the result of gene duplication through evolutionary time, probably because of inhibitors and toxins that they encounter in their food. The sequencing method allows us to quantitate expression of each cysteine cathepsin, and overall expression is similar in both insects. Changes in conserved sequences may indicate difference in substrate or enzyme specificity. In the course of this study, we discovered a new group of enzymes with atypical structures, thus far not found in other organisms. These data will help in the design of new biological control products for stored product beetles.

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Vaccines for prevention of bluetongue and epizootic hemorrhagic disease in livestock – a North American perspective

Authors: D.S. McVey, N.J. MacLachlan

Submitted to: Vector-Borne and Zoonotic Diseases
Bluetongue (BT) and epizootic hemorrhagic disease (EHD) are non-contagious, insect transmitted diseases of domestic and wild ruminants caused by related but distinct viruses. Both BT (BTV) and EHD (EHDV) viruses cause hemorrhagic fevers in susceptible ruminants; however BT is principally a disease of domestic livestock whereas EHD is principally a disease of certain species of wild, non-African ungulates, notably White-tailed deer. The live-attenuated (modified live virus [MLV]) vaccines available in the United States (U.S.) for use in small ruminant livestock do provide good protection against clinical disease following infection with the homologous virus serotype. Although there is increasing justification that use of MLV vaccines should be avoided if possible, these are the only vaccines currently available in the

U.S. Specifically, MLVs are used in California to protect sheep against infection with BTV serotypes 10, 11 and 17, and a MLV to BTV serotype 10 is licensed for use in sheep throughout the U.S. These MLV vaccines may need to continue to be used in the immediate future for protective immunization of sheep and goats against BT. There are no currently licensed vaccines available for EHD in the U.S. If there is a need to rapidly develop a vaccine to meet an emerging crisis associated with either BTV or EHDV infections, development of an inactivated virus vaccine in a conventional adjuvanted formulation will likely be required. With two doses of vaccine (and in some instances just one dose), inactivated vaccines can provide substantial immunity to the epizootic serotype of either BTV or EHDV. This strategy is similar to that used in the 2006-2008 BTV serotype 8 outbreaks in northern Europe which provided vaccine to the field within 2 years of the initial incursion (by 2008). There are significant gaps in our scientific knowledge and available countermeasures to control an outbreak of orbivirus-induced disease, whether BT or EHD. It is concluded that a coordinated national research strategy to achieve a more optimal vaccine profile should be developed.

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Susceptibility of adult midges to insecticidal sugar baits

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Submitted to: Medical and Veterinary Entomology
Biting midges transmit viruses that result in livestock and wildlife sickness and death, therefore reducing the disease transmitting insect population insecticidal sugar baits will help reduce risk to animals. Various commercial insecticide products were mixed with 10% sugar water to determine if midges would feed on the toxic solutions. Midge mortality was measured at 1, 4, 10, and 24 hours to determine how well the insecticidal sugar baits functioned. Several pyrethroid mixes worked very well within the first hour. This new technique to kill midges has several advantages one of which is the use of new pesticides to combat the evolution of insecticide resistance exotic biting midge transmitted pathogens. Here we outline current knowledge of vector ecology and control options for North American biting midges and delineate research recommendations aimed to fill knowledge gaps and lead to the design and implementation of control tactics.

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Feeding status alters biting midge (*Culicoides sonorensis*) photoattraction

Authors: D. Snyder, N. Cernicchiaro, L.W. Cohnstaedt
Submitted to: Medical and Veterinary Entomology
The biting midge, *Culicoides sonorensis* Wirth and Jones (Diptera: Ceratopogonidae), vectors both livestock and wildlife diseases. Midge fieldwork depends heavily on light traps for collection. Little is known regarding midge vision, photoattraction, or light spectra preferences. A biological light assay arena was constructed and light-emitting diodes (LEDs) of various light spectra were used as light sources to evaluate midge photoattraction. A comparison of responses to light spectra indicated the highest proportion of *C. sonorensis* were attracted to UV light, and that midges were able to distinguish 10 nm differences in light wavelengths. Ultraviolet light intensity was also evaluated and *Culicoides* showed a higher level of attraction to stronger intensities. Midges exhibited sugar seeking and escape behaviors under different sugar supplementation scenarios before and during the experiment, which can be attributed to lights of 355 nm and 365 nm wavelengths. Based on the results of this study, *C. sonorensis* attraction to light traps can be improved by using more intense (brighter) lights of 355 or 365 nm wavelengths.
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Interaction of sorghum tannins with wheat proteins and effects on *in vitro* starch digestibility in wheat flour tortillas

Authors: K.L. Dunn, L. Yang, A. Girard, S.R. Bean, J.M. Awika
Submitted to: Journal of Agricultural and Food Chemistry
Excess calorie intake and associated disease risks are a major problem in many parts of the world. Carbohydrates contribute the most calories in the diet, making starch-based foods a logical target for improvement. This study investigated the potential interaction of sorghum bran proanthocyanidins (tannins, PA) with proteins and starch during wheat flour tortilla processing, and impact on *in vitro* starch digestibility. Refined wheat flour tortillas were substituted at 10, 15, and 25% (baker's) with brans from wheat and white (low in phenols), brown (high in PA), and black (high in monomeric phenols) sorghum. Dough phenolic profile, starch digestibility, and protein solubility and digestibility were evaluated during tortilla processing and storage. Dough formation drastically decreased extractable phenol content in brown sorghum bran dough ($\geq 58\%$) compared to non-tannin bran treatments ($\leq 18\%$). A large decrease in extractability of polymeric PA was responsible for the decrease in phenols in the brown sorghum dough. The PA in brown sorghum bran interacted with proteins during dough mixing to significantly increase insoluble proteins (IP) (460 mg/g protein) compared to other sorghum bran treatments (330 mg/g protein). The brown sorghum bran (at 25%

substitution) tortillas had significantly ($p < 0.05$) higher slow digesting starch and lower rapidly digesting starch than all other bran treatments, suggesting the PA-protein interactions may have slowed starch digestibility. *In vitro* protein digestibility of the brown sorghum bran tortilla was also the lowest, but not significantly different from the black sorghum bran treatment. Overall, evidence suggests that the sorghum PA significantly interact with wheat proteins during dough development in ways that may impact starch digestibility in wheat-based products. Contact Scott Bean, telephone 785-776-2725, email Scott.Bean@ars.usda.gov

Genetic characterization of epizootic hemorrhagic disease virus strains isolated from cattle in Israel

Authors: W.C. Wilson, M.G. Ruder, E. Klement, D.C. Jaspersen, H. Yadin, D.E. Stallknecht, E. Howerth
Submitted to: Journal of General Virology
A bluetongue-like disease reported in Israeli Cattle was found to be associated with Epizootic Hemorrhagic Disease Virus (EHDV). EHDV had not been previously reported in Israel. Genetic analysis was done to determine the potential origin of this virus. The virus genetics was related to those from Africa and Mediterranean region. This finding also supports the hypothesis that EHDV entered Israel during 2006 and was not present there before this outbreak.
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