

## Fire Ant Initiative Action Plan – FY 2006-2007

Maximum 500 words, double-spaced, type size at least 12 points, do not exceed boundaries. Include statement on anticipated outcomes.

IFA females fly, locate clouds of males, mate once and then seek a place to land. They remove their wings (wing casting), lay a few eggs and their wing muscles begin to degenerate (colony initiation). Wing muscle degeneration yields resources used to nurture her first larvae through to adult workers. We propose to isolate the factor(s) that initiates female wing muscle degeneration. Earlier we investigated the roles of several factors that initiate flight muscle breakdown and wing casting. Our results showed that flight muscle breakdown is by an apoptosis event, not necrosis. Apoptotic cells were identified by 3' end labeling fragmented nuclear DNA (TUNEL Assay). Using this bioassay to measure apoptosis early, we showed that JH initiates wing casting, but not muscle degeneration. We also found that apoptotic nuclei only occurred following mating and we were able to initiate apoptosis by artificially inseminating females. However, the same factors may also initiate egg laying. Since the initial methods are the same we will examine this possibility as well.

Davis et al. (1989) found that hemolymph from queens following a mating flight would initiate muscle histolysis when injected into non-flight (non-mated) queens. Based on this we isolated one novel protein from the hemolymph of newly collected mated queens that were not present in the hemolymph of either virgin queens or in queens two days post-mating. In a reverse genetics approach we were able to isolate a 252 bp DNA fragment corresponding to this protein that is homologous to the cysteine protease inhibitor (CPI). The expression of putative CPI was monitored at transcriptional levels to establish its presence. A significant increase in the expression of putative *CPI* was observed in mated as compared to alate virgin queens from day 0 post-mating and continued to increase for another three days; on day 7 the expression vanished. We propose three pathways to determine if these or yet another compound is involved in apoptosis. One is isolating the hemolymph apoptosis initiation factor of Davis through traditional chemical bioassay driven purification. The second is to use RNA interference (RNAi) method to determine the functional role(s) of putative fire ant queen CPI gene in the initiation of apoptosis. The third is to isolate and identify the component(s) of the male seminal fluid that initiate flight muscle histolysis. The results will provide molecules that control muscle apoptosis and initial reproduction that will provide new approaches to IFA management.