

ENTOMOLOGY

Chairman: ROBERT PINGER
Department of Physiology and Health Science, Ball State
University, Muncie, Indiana 47306

Chairman-Elect: R. BRUCE CUMMINGS
Indiana Department of Natural Resources, 6311 North
County Road 50 West, LaPorte, Indiana 46350

ABSTRACTS

The Influence of Nutrient Levels on the Colonization of Tires by Mosquitoes. WILLIAM J. BERRY, Vector Biology Laboratory, University of Notre Dame, Notre Dame, Indiana 46556. — Previously unexploited tires were placed in tireyards and woodlots in shaded and exposed areas. The rate of colonization of the new habitats by tire inhabiting mosquitoes was determined by sampling the tire contents from June through September. The rate of colonization was found to differ greatly between species and also was influenced by the amount of exposure to the sun and the amount of leaf litter present in the tire. *Culex restuans* was found to be the first species to exploit the new habitat; *Aedes atropalpus* and *Aedes triseriatus* were slower to colonize and occurred in fewer numbers than *Culex restuans*.

Electrophoretic Separation of Northern Indiana Culex Species. BARTHOLOMEW G. CORSARO and LEONARD MUNSTERMANN, University of Notre Dame, Notre Dame, Indiana 46556. — The Saint Joseph County (Indiana) Mosquito Abatement Program conducts routine surveillance for nuisance and vector mosquitoes with sixteen New Jersey light traps located in major population centers throughout the county. The primary purpose of the project is to assess the risk of Saint Louis Encephalitis in the county. Unfortunately, *Cx. pipiens*, the major vector of SLE, is virtually impossible to separate from adult *Cx. restuans* because characteristic scales are rubbed off either by age or the trapping device.

Starch gel electrophoresis was examined as a method to separate these species. Electrophoresis was performed on adult specimens of *Cx. pipiens*, *restuans*, *territans*, and *salinarius*, that were collected and identified as larvae.

Twenty-seven enzymatic assays were attempted, of these, seventeen produced repeatable banding patterns. Six loci (IDH, GPI, ME, AK, ACO, ODH) separated *Cx. restuans* from *Cx. pipiens* and *salinarius*; while only one loci (ME) separated *Cx. salinarius* from *Cx. pipiens*. No enzymes separated *Cx. territans* though this is unimportant since it possesses reliable morphological characters. Most importantly, *Cx. pipiens* could reliably be separated from *Cx. restuans*.

This information was used to determine the proportion of each *Cx.* species in five of the sixteen light traps in the surveillance program. A CDC net bag was attached to the New Jersey traps once a week from June through August 1982. The *Culex* mosquitoes collected were identified by starch gel electrophoresis using the enzyme systems mentioned above. These techniques allowed us to determine the density of *Cx. pipiens* in relation to *Cx. restuans*, and the time of first appearance of *Cx. pipiens* in the light traps.

On the Genetic Basis of Non-reciprocal Fertility among Members of the Aedes (Stegomyia) scutellaris Group (Diptera, Culicidae). VAS DEV and KARAMJIT S. RAI,

Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556. — *Aedes (Stegomyia) scutellaris* group is composed of over 30 species. Of these, *Aedes malayensis* and *A. alcasidi* are very closely related species separable mainly by the distinct claspette of the male genitalia. Interspecific crossing experiments revealed that there existed only a unidirectional compatibility between these two species. 100% of *A. malayensis* females were inseminated by males of *A. alcasidi* and gave viable progeny. However, in the reciprocal cross, 100% of *A. alcasidi* females were inseminated but all eggs laid were sterile.

Hybrid progeny obtained from the cross using *A. malayensis* females and *A. alcasidi* males were backcrossed to either parent. *A. malayensis* females inseminated by hybrid males laid viable eggs. However, eggs laid by the *A. alcasidi* females inseminated by the hybrid males, were all sterile. The hybrid females laid viable eggs irrespective of the type of species males use. Thus, it is evident that it is always the *A. alcasidi* females which lay sterile eggs in the interspecific crosses.

Of several possibilities, the chromosomal basis of this unidirectional compatibility appears unlikely for the following reasons. *A. alcasidi* females when inseminated by hybrid males should be able to recognize its homologous chromosomes (barring crossing over) resulting in some degree of fertility depending on the number of chromosomes involved. Besides, a proportion of females, recovered from the backcross (hybrid females \times *A. alcasidi* males), when further backcrossed to *A. malayensis* males, are expected to lay sterile eggs but all laid viable eggs based on the 35 families raised. From this crossing scheme, it appears that the genetic basis of unidirectional fertility is of cytoplasmic origin and independent of nuclear chromosomes.

Ice Nucleating Proteins from the Freeze Tolerant Queens of the White Faced Hornet, *Vespula maculata*. JOHN G. DUMAN, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556. — In late summer 100-200 new queens are produced in the nest of the white faced hornet, *Vespula maculata*. After being inseminated these future queens overwinter individually in logs, etc. During this overwintering period they are freeze tolerant (able to survive the freezing of their extracellular fluids). In spite of the presence of high levels of the cryoprotectant glycerol, the supercooling points of the overwintering queens are quite high (about -5°C) because of the production (beginning in the pupal stage) of ice nucleating proteins. These hemolymph proteins function to inhibit supercooling. This insures that ice formation occurs at fairly high temperatures and that it is initiated in the hemolymph. This is important because freezing after extensive supercooling can lead to intracellular ice formation which is lethal, even for freeze tolerant insects.

An ice nucleating protein was purified from the hemolymph of pupal *V. maculata* queens using ion exchange (DEAE-Sephadex), gel filtration (LKB-Ultogel-ACA-54) and preparative polyacrylamide gel electrophoresis. This is the first ice nucleating protein to be purified. The protein has a molecular weight of 74,000, as determined by SDS-PAGE. The amino acid composition of the protein is interesting because it contains ~ 20 mol % glutamic acid and/or glutamine. This along with the other hydrophilic amino acids (Asp, Lys, Asn, Arg, Ser, Thr) makes the protein quite hydrophilic. Hydrophilic amino acids account for 54% of the amino acids present in the protein. It is likely that the hydrophilic nature of the protein allows it to order water in a special way so that small clusters of water molecules (embryo crystals) are aggregated around the protein. Once these embryo crystals reach a critical size they then seed the solution.

Hormonal Regulation of Antifreeze Protein Production in Larvae of the Beetle, *Dendroides canadensis*. KATHLEEN L. HORWATH and JOHN G. DUMAN, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.—Larvae of the beetle *Dendroides canadensis* accumulate protein antifreezes during the winter which depress their hemolymph freezing and supercooling points, thereby enhancing their overwintering survival. This study describes experiments investigating the endocrine control of these antifreeze proteins. *D. canadensis* which were collected in the early fall, prior to the initiation of cold hardening processes, were treated with either 3.3 or 6.6 μg juvenile hormone I topically in acetone and maintained for 21 days under normally non-inductive acclimation conditions (16 light/8 dark, 20°C). Hormone treated animals significantly elevated the levels of antifreeze protein in their hemolymph compared to those of acetone treated and untreated controls or animals measured on the day of collection.

D. canadensis treated with the anti-JH compound precocene II (P2) in acetone for 24 hr at a concentration of 20 $\mu\text{g}/\text{cm}^2$ (a dose below the LD_{50} for 'behavioral' survival) and then maintained under acclimation conditions conducive to antifreeze protein production (8 light/16 dark, 20°C) for 2 weeks failed to elevate levels of antifreeze. Acetone treated control animals accumulated a significant concentration of antifreeze protein. *D. canadensis* were also treated with 20 and 150 $\mu\text{g}/\text{cm}^2$ P2 (a dose below the LD_{50} for 'gross' survival) followed by acclimation to short (8 hr) photoperiod at 10°C. All animals receiving the higher P2 dosage failed to elevate antifreezes while only 42.9% of the individuals treated with the lower dosage initiated antifreeze protein production. In contrast, over 80% of untreated and 70% of acetone treated controls responded to these inductive acclimation conditions by elevating antifreeze concentrations.

These results indicate that juvenile hormone participates in the seasonal control of antifreeze protein production in *Dendroides canadensis*. Since this species does not enter a diapause state prior to or throughout the winter this is the first evidence establishing a direct hormonal mechanism involved with insect cold hardness.

This research has been supported in part by a grant from the Indiana Academy of Science to K.L.H.

Evaluation of a Method of Estimating Yields of Alfalfa under Insect Stress. MARK A. ZAJAC and M. CURTIS WILSON, Department of Entomology, Purdue University, West Lafayette, Indiana 47907.—Investigators have suggested that alfalfa yields may be accurately estimated to within 10% of actual yields using plant height as the sole estimating variable. They developed a fourth order polynomial equation relating crop height to yield. This equation was assumed to be accurate in full stands of alfalfa (35 or more stems per square foot.).

Data from research conducted over the past 10 years in Indiana on the effects of the potato leafhopper, *Empoasca fabae* (Harris), on alfalfa yields show a high correlation between insect populations and crop height. Consequently, yields estimated through the formula and potato leafhopper populations were also highly correlated. However, insect populations showed a low correlation with harvested yields. Crop height and harvested yields also showed little correlation. Data from 11 field studies support the conclusion that crop height is of little value in estimating yields of alfalfa under insect stress.