I-2001
Optimization of Feeding Strategies for Heterologous Protein Production in Cultures Using the Insect Cell-Baculovirus System. J.-C. Drugmand, J.-F. Michiels, Y.-J. Schneider, and S. N. AGATHOS. Unit of Bioengineering, University of Louvain, Louvain-la-Neuve, B-1348, BELGIUM. E-mail: agathos@gebi.ucl.ac.be

Insects are capable of resisting toxic effects of insecticides through a variety of defence mechanisms. In many cases an important route of resistance is that prior to the insecticide action on the target sites, the insecticide molecules must penetrate in the insect body and resist rapid clearance via the insect gut into the faeces. In this study, we have assessed the functionality of ex vivo midgut sacs of the important caterpillar pest, the cotton leafworm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), by determination of the transport kinetics through the barrier composed of the midgut epithelium and the peritrophic membrane. Here we used an important group of insect growth regulators, namely the chitin synthesis inhibitors. Data using midgut sacs from last (6th) larvae were compared with the in vivo profile in the body tissues and excrements of last instars. For the obtained absorption and excretion kinetics, we calculated mass differential equations in a two- and three-compartment model.

I-2002
Application of Inter-Simple Sequence Repeats (ISSR) to Insect Cell Lines: Identification at the Clonal and Tissue-specific Level. J. J. GRASELA, A. H. McIntosh, C. L. Goodman. USDA, ARS, Biological Control of Insects Research Laboratory, 1503 S. Providence Rd., Columbia, MO 65203-3535. E-mail: graselajj@missouri.edu

Inter-simple sequence repeat (ISSR) primers designed to anneal to microsatellites were used to obtain DNA fingerprint profiles to distinguish between 16 established insect cell lines derived from an assortment of lepidopteran, dipteran, and coleopteran species. Three different levels of cell line comparison were made: (1) between parents and their clones; (2) between cell lines derived from different tissues from the same species; and (3) between cell lines derived from different insect species. Of the 16 repeat oligonucleotide primers employed in this study, nine primers generated several unique markers to distinguish between parental cell lines and their clones. Four of the 16 primers also generated DNA profiles with a number of unique bands, enabling the distinction between cell lines derived from specific tissues from the same species. In addition, ISSR-generated DNA profiles provided the greatest number of unique markers to easily distinguish between insect cell lines derived from different species.

I-2003
Does Apoptosis Play a Role in the Growth and Metamorphosis of the Lepidopteran Midgut? RAZIEL S. HAKIM. Howard University College of Medicine, Washington, DC 20059. E-mail: rhakim@mac.com

Apoptosis plays a central role in development, disease control, and removal of host cells no longer needed. This process tends to be targeted towards specific cells. During midgut growth and differentiation in the lepidopteran Manduca sexta there are several times when apoptosis might be involved in the control of cell number. First there is an enormous enlargement of the population of stem cells and their progeny in each larval instar-- the controls which limit the final number of such cells are unknown; Second there is the possibility that the mature goblet or columnar cells already present in each instar, turn over, despite our not having seen evidence for this process histologically. Lastly, as part of metamorphosis, there is a delamination of remnant larval epithelium, and replacement by a prepupal epithelial population. For these reasons apoptosis was tested for in these three contexts... Annexin tests were carried out on stem cells and undifferentiated progeny collected from larval epithelium, and TUNEL tests were carried out on histological sections of larval and prepupal midgut. Only the remnant larval epithelium which delaminates from the prepupal midgut demonstrates apoptosis. Neither larval stem cells, nor the mature goblet or columnar cells undergo programmed cell death during the course of an instar or subsequent molt.
An Assay System for Determining the Toxicity of Flavonoids against Insect Cells In Vitro. J. L. GRINGORTEN, M. Abou-Zaid, and G. Caputo. Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON P6A 2E5, Canada. E-mail: lgringo@nrcan.gc.ca

Flavonoids are present in plant foliage in various combinations and concentrations, some of which provide a means of natural defense against defoliating insects. As part of an investigation into their mode of action, we developed a cell system to examine their cytotoxicity in vitro. We describe a procedure for dissolving freeze-dried ethanolic leaf extracts and purified compounds in dimethyl sulfoxide and testing their activity directly against cells suspended in agarose, while avoiding solvent toxicity. Fifteen purified compounds and ten leaf extracts were tested against a lepidopteran cell line, CF-1, in this system and their cytotoxic activities determined. Threshold doses of flavonoids that were toxic to CF-1 ranged from 0.25 \( \mu \text{g} \) (quercetin and morin) to 16 \( \mu \text{g} \) (\(^{(+)}\) catechin). The range for leaf extracts was 0.13 \( \mu \text{g} \) (mulberry, Morus alba L.) to 2 \( \mu \text{g} \) (scotch pine, Pinus sylvestris L.). The activities of several leaf extracts were markedly enhanced when their pH was increased to a level similar to that of the larval lepidopteran midgut. Sugar maple (Acer saccharum Marsh.) toxicity increased more than 16-fold at the higher pH. The pH effect was abolished in the presence of larval gut juice.