## A New Type of Solid, Semi-Solid, and Semi-Liquid Arthropod Artificial Diets Using Colloids to Replace



# **Gelling Agents**

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Artificial production of insects has been done by the use either solid, gel, or liquid diets Atallah 1966, Bosse et al. 1981, Cohen 2004, Rojas and Morales 2008, Shaver and Raulston 1971, Villacorta 1985, and White et. al. 2000. Solid diets rely mostly on the use of agar which is a gelling agent commonly used in microbiology. Agar-based artificial diets require special temperature conditions for mixing and dispensing. These conditions often conflict with temperature requirements of some of the most important nutritional ingredients (mostly vitamins) to remain intact. Semi solid and liquid diets may not require heat during their preparation; but they are prone to microbial contamination due to water excess and as a consequence the concentration of antimicrobials in the diets has to be increased which is also detrimental to arthropods. The objective of this work was to develop new diet formulations for economical important arthropods using a colloidal agent to eliminate heat, reduce the use of deleterious chemicals, preparation time, and improve storage.

## **Materials and Methods**

Insects. Helicoverpa virescens, Lygus hesperus, L. lineolaris, were obtained from laboratory colonies reared at USDA ARS JWDSRC, Stoneville MS; Trichoplusia ni from C-Perl Co., Coleomegilla maculata from corn fields around JWDSRC; and Tetranychus urticae, and Phytoseiulus persimilis from Syngenta Bioline.

Diet preparation. The control diets were prepared following the protocols provided by the cooperators, the colloidal formulations were based on the chemical analyzes of natural hosts and control diets. Once the chemical formulations were established, water content was adjusted to resemble that of the natural host and the colloidal agent was added to a ratio to obtain the desired consistency ranging from semi-liquid to solid (Rojas and Morales, 2008).

Rearing. Diets for sucking mouth part arthropods were encapsulated in Parafilm®, for chewing insects diets was presented with out protection.



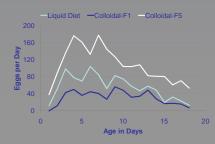
From left to right: Western tarnished plant bug (*Lygus hesperus*), two-spotted spider mite (*Tetranychus urticae*), predatory mite (*Phytoseiulus persimilis*), spotted lady beetle (*Coleomegilla maculata*), and tobacco budworm (*Helicoverpa virescens*) feeding on different versions of the colloidal artificial diet.

**Bioassays.** Equal number of eggs per species were placed in plastic containers. Treatments were fed every 3 days for plant bugs and mites, every other day for C. *maculata*, and once for the tobacco budworm. Controls were fed with their regular food source. Cohen diet for plant bugs, red bean leaves for spider mites, spider mites for predatory mite and modified Raulston diet for tobacco budworm. All bioassays were performed in Percival Environmental chambers at 27 °C, 67% RH and 16:8 L:D cycle, with the exception of mites in which the temperature was set at 26 °C.



ation containers, *Tetranychus urticae* evaluation dishes, *maculata* boxes for larval development e<u>valuation, and</u> From left to right: *Lygus hesperus* and *L. lineolaris* evalu toseiulus persimilis evaluation containers, Coleomegilla maculata coverpa virescens and Trichoplusia ni flasks for fecundity evaluation.

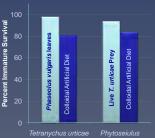
The physical consistency of these colloidal artificial diets can be adjusted from semi-liquid to solid by varying the water content. The colloidal components do not need to be heated during the mixing process allowing sensitive nutrient ingredients to be mixed together. The colloidal particles provide a substrate that enhances extra-oral digestion making these type of diets suitable for a large range of predatory arthropods. Colloidal diets formulations have been develop with satisfactory results for a variety of arthropods including the ones presented in this study. Solid formulations were developed for the two lepidopterous species.



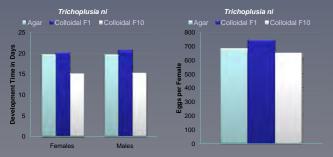
Lygus lineolaris fecundity decreased when switched from the liquid to the colloidal diet. However their fecundity increased to twice the levels of those of the liquid diet by the fifth generation in colloidal diet. Egg viability was significantly higher in the colloidal diet (86.53 and 91.9% in the F1 and F5, respectively) as compared to the liquid diet (59.57%) The two Lygus species have been reared in a semi-liquid colloidal diet for over 10 generations. Development time decreased significantly in the 10<sup>th</sup> generation as compared to the first generation on colloidal diet.

The diet formulation for C. maculata has a semi-solid consistency, but adults and larvae feed directly on the diet. This coccinellid has been reared in the new artificial diet for 3 generations without changes in development time and fecundity. The development time of C. maculata was 21.43 ± 2.1 days at 27 ± 1°C in the progeny from field collected adults developing on the colloidal diet and survival from egg to adult was 24.72% .

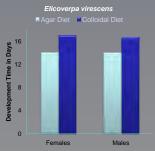
Survival of both T. urticae and P. persimilis from egg to adult was over 80% when grown in the colloidal diet. The loss of survival was less than 10% in P. persimilis feeding on the colloidal diet as compared to feeding on natural prey. Difficulties remain in P. persimilis in vitro rearing as adults do not oviposit in the absence of prey. Oviposition has been induced by the addition of aqueous extracts from T. urticae.



Development time of T. ni was slightly longer in the first generation on colloidal diet as compared to the agar base diet, but it decreased by the 10<sup>th</sup> generation to significantly shorter levels as compared to the agar-based diet. Fecundity was not significantly affected by diet formulation in T. ni. A slight reduction was observed from the first to the tenth generation, but this difference was not statistically significant.



Development time of H. virescens reared on colloidal diet was significantly longer than those reared on an agar based diet, but only the first generation of *H. virescens* on colloidal diet was studied.



The elimination of agar and excess of water from diet formulations significantly simplified the mixing of these arthropod artificial diets. Dispensing colloidal diets does not require temperature controls since it remains liquid, semi-solid, or solid at room temperature. Diet consistency is controlled by the colloid to water concentration ratios. Thus reducing the complexity of the mechanized process for mixing and dispensing artificial diets into rearing containers.

Carlos Blanco (former SIMRU researcher), Brenda Woods, Terry Johnson, Matthew McDaniel, Archie Clark nd Derrick Jones for providing and maintaining control stock colonies. Greg Walker and Leon Hicks for C nac. field specimen collection, BCPRU, USDA, ARS MSA. Daniel Cahn and Richard GreatRex, Syngenta ioline CRADA partner.

- References Cited tailah, Y. H. and L. D. Newsom. 1966. Ecological and nutritional studies on Coleomegilla maculata De Geer (Coleontera: Coccinellidae). I. The development of an artificial diet and a laboratory rearing technique
- A. Veerman. 1981. A meridic diet for the two-spotted spider mite ina, Tetranychidae). Med. Fac. Landbouww. Rijksuniv. 46: 499-502 Id mixer solid or semi-solid ieres LLC (Coleoptera: Coccinellidae), I. The develop J. Econ. Entomol. 59: 1173-1179. Th. C.L.P. S. Van der Geest and A. Veermai *Tetranichus urticae* Koch (Acarina, Tetran Cohen AC. 2004. Insect diets: science and M. G. and J. A. Morales. 2008. Cold mixer s AGAR. Invention disclosure accented bur

- 2008., serial N haver TN, Raulston J mer. 64: 1077-1079. illacorta A. 1985. Dieta (Coleontor
- Oct. Of Not. A 1985. Dieta meridica para criacao de sucessivas geracæs de Hypothenemus hampei Ferral (Coleoptera: Scolytidae). Anais da Sociedade Entomologica do Brasil 14: 315-319.
  J. H., L.A. Stauffler, and K.A. Gallagher. 2000. Methods for rearing insects, mites, and other bene organisms. U.S. patent No. 6,129,935.