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Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) powder to enhance artificial diet formulations for *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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HIGHLIGHTS

- We developed artificial diets for the predatory lady beetle *Coleomegilla maculata*.
- We compared artificial diet formulations to a natural food mix.
- We supplemented meridic artificial diets with dry extracts of the factitious prey *Tenebrio molitor*.
- Meridic artificial diets were inferior to the control and to those including *T. molitor* extracts.
- *Coleomegilla maculata* fed artificial diets with *T. molitor* extracts produced more progeny and their doubling time was shorter than those fed the control diet and the diets lacking insect components.

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ABSTRACT

The predatory lady beetle *Coleomegilla maculata* has potential to control several arthropod pests on crop plants in greenhouses and high tunnels. However, an effective artificial diet is needed in order to mass produce *C. maculata* in sufficient quantities for augmentative releases. The objectives of this study were to develop a semi-solid insect-free artificial diet, evaluate the diet effects on *C. maculata* fitness, and determine if adding extracts (consisting of dry powder of whole pupae) of the yellow mealworm, *Tenebrio molitor*, could improve the suitability of the diet formulations and the life parameters of *C. maculata*. Although *C. maculata* completed development and reproduced on two meridic artificial diet formulations (M1 and M2), neither one was as effective as a control diet mix consisting of *Ephestia kuehniella*, *Artemia* sp. eggs, and bee pollen. Incorporation of *T. molitor* extracts into the diet formulation (5 and 7% in diets T1 and T2, respectively), significantly improved *C. maculata* larval survival, shortened post-embryonic development time, increased fecundity and egg viability as compared to the meridic diet formulations. Adults feeding on diets T1 and T2 (containing *T. molitor* extracts) produced more eggs and had a better survival than those feeding on the control diet mix. Also, egg viability was significantly higher in diets T1 and T2 than in the control. Demographic parameters showed that the overall fitness of *C. maculata* fed the meridic diet containing *T. molitor* extracts was superior to beetles fed the control mix. The role of feeding stimulation by *T. molitor* added components as responsible for the diet improvement is discussed.

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1. Introduction

Coleomegilla maculata De Geer (Coleoptera: Coccinellidae) inhabits landscapes in North, South, and Central America (Gordon, 1985; Munyaneza and Obrycki, 1998; Krafsur and Obrycki, 2000). It is a generalist predator of aphids, lepidopteran eggs and larvae, chrysomelid eggs and larvae, spider mites, and other soft-bodied prey (Michaud and Jyoti, 2008; Hodek and Evans, 2012), and can complete development on plant pollen (Smith, 1960, 1965; Michaud and Grant, 2005; Lundgren et al., 2011). There is interest in using this ladybird beetle for augmentative biological control of plant pests in protected culture, i.e., high tunnels, greenhouses, plantscapes and interiorscapes (Riddick et al., 2014a, 2014b, 2014c). Its polyphagous tendencies are an advantage when there are multiple pest species on the same crop plant. Another advantage is that in the absence of prey, *C. maculata* can survive on plant material such as pollen and nectar.

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Mass production of *C. maculata* is necessary for augmentative biological control to provide enough quantities of immature and/or adult stages to control pests on plants on a commercial scale. One approach to facilitating mass production of *C. maculata* is through the provision of inexpensive, nutritious artificial diets that promote development, growth and reproduction of *C. maculata* over consecutive generations. Artificial diets devoid of insect components, or meridic diets (Grenier and De Clercq, 2003), would greatly reduce costs associated with rearing *C. maculata*, in lieu of natural prey, because natural prey (such as most aphid species) often require live host plants as food (Hagen, 1987; Riddick and Chen, 2014). No such standalone, arthropod-free artificial diet is currently available for lady beetles, but research has been ongoing (Smirnoff, 1958; Atallah and Newsom, 1966; Silva et al., 2010; Sighinolfi et al., 2013).

Exploring the effectiveness of artificial diets containing plant protein, rather than insect protein, is another line of research with potential. In efforts to identify a highly effective artificial diet, Riddick et al. (2014c) tested the usefulness of a powdered formulation of *Spirulina platensis* (Nordstedt) Geitler (Cyanophyceae: Phormidiaceae), a protein- and vitamin-rich cyanobacteria microalga, as a supplement to a suboptimal meridic artificial diet for *C. maculata*. Unfortunately, *S. platensis* was not a satisfactory supplement; since it did not improve the quality of the suboptimal artificial diet for *C. maculata* (Riddick et al., 2014c).

Difficulties in developing effective meridic diets arise from nutritional requirements that are particular to insects. For example, insects require higher quantities of the amino acids proline and tyrosine than vertebrates (Wigglesworth, 1972; Carter et al., 2006; Lundgren, 2009). Levels of these two amino acids present in vertebrate and vegetable protein are not adequate for insect nutrition and must be added to meridic diet formulations (Morales-Ramos et al., 2014). Adequate levels of these amino acids are found in the protein of natural prey and non-prey food such as pollen (Lundgren et al., 2005). Additionally, artificial diets that contain insect components are useful when predators depend on chemical cues to stimulate feeding (De Clercq, 2004). Morales-Ramos et al. (2014) suggested that insect protein may be added to artificial diet formulations from insect species that are produced commercially. The yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is commercially available in the United States, and some North and South American, European and Asian countries (van Huis et al., 2013). In some countries *T. molitor* powder extracts are commercially available, which could be easily incorporated into artificial diet formulations (Morales-Ramos et al., 2014).

The objectives of this study were: (1) to compare the biological and demographic parameters on *C. maculata* fed semi-solid meridic artificial diets with a control mix consisting of natural ingredients and (2) to determine if adding powdered pupae of the yellow mealworm, *T. molitor*, to diet formulations could improve their suitability and the life parameters of *C. maculata*. As such, we tested the hypothesis that supplementing a suboptimal meridic artificial diet with dry extracts from *T. molitor* improves the biological and demographic parameters of *C. maculata*.

2. Materials and methods

2.1. Origin of colonies and maintenance

The stock colony of *C. maculata* was initiated from field collections conducted in the spring and summer of 2010 in Stoneville, Mississippi. Adults were maintained in a 2-section modular cage, which provides an upper space for feeding and a lower space for oviposition (Fig. 1A). The upper section was constructed from plastic containers (312 × 230 × 102 mm) (Product 295C, Pioneer Plastics Inc., North Dixon, KY), which were modified by adding 10 screened windows (nylon screen with 500 μm openings) on the sides (30 mm diam.) and 8 openings on the cover (52 mm diam.) that could be used as windows or water sources (Fig. 1A). The bottom section was constructed from cylindrical plastic containers (191 × 202 mm diam.) (Product 289C, Pioneer Plastics Inc., North Dixon, KY) and modified by adding a side opening with screwed cap to allow the exchange of oviposition substrate (Fig. 1B). The stock colony was fed with a combination of food sources including Entofood® (Koppert Biological Systems Inc., Howell, MI), ground bee pollen granules (product 2530, NOW Foods, Bloomingdale, IL), *Lygus hesperus* (Knight) (Heteroptera: Miridae) eggs, ground lyophilized *T. molitor* pupae, honey, and a meridic artificial diet as supplement. Water was provided by saturated water crystals of cross-linked potassium polyacrylate and polyacrylamide copolymer (T-400, Terawet Inc., San Diego, CA) placed in screened devices on the cover of the cage. The oviposition substrate consisted of stacked and folded paper sheets (Kimwipes®, Kimberly-Clark LLC, Roswell, GA) placed standing vertically inside the bottom section of the cage. Egg masses were cut from the paper sheets and deposited in Petri dishes every day for development. First instars were transferred to cages constructed from square hinged plastic boxes (334 × 233 × 50 mm) (product 700C, Pioneer Plastics Inc., North Dixon, KY) and modified by

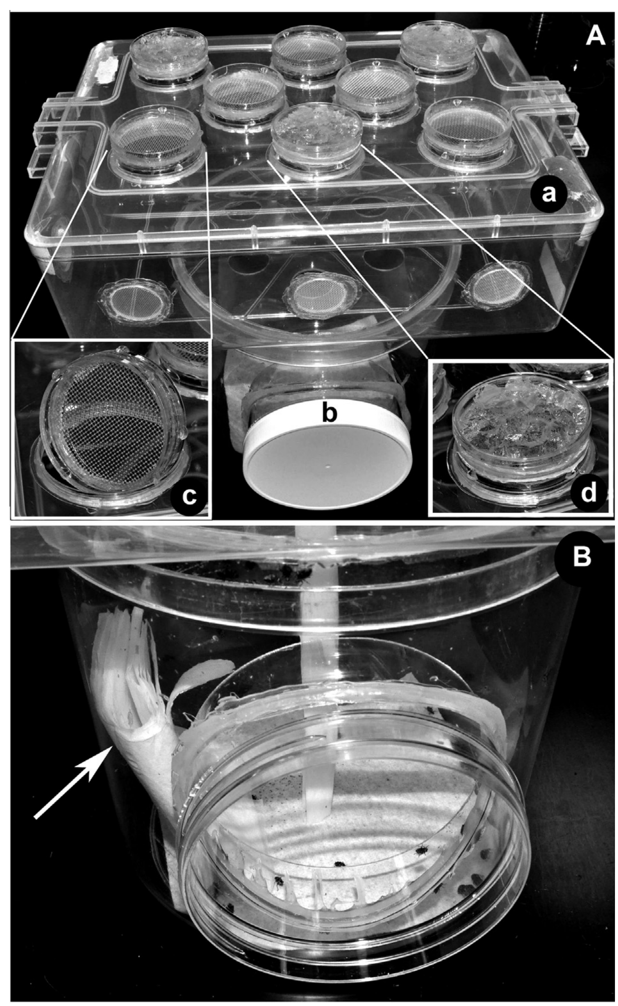

Fig. 1. Adult cage design for *C. maculata* rearing. (A) Fully assembled cage showing the upper section (a), lower section (b), detachable cover windows (c), and water sources with saturated water-absorbing polymer (d). (B) Lower cage section with open access showing a stack of paper sheets as an oviposition substrate (arrow).