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Artificial tritrophic exposure system for environmental risk analysis on aphidophagous predators

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ABSTRACT

We evaluated an artificial tritrophic exposure system for use in ecotoxicological evaluations of environmental stressors on aphidophagous predators. It consists of an acrylic tube with a Parafilm M sachet containing liquid aphid diet, into which can be added environmental stressors. Immature *Cycloneda sanguinea, Harmonia axyridis* and *Chrysoperla externa,* and adult *H. axyridis* were reared on *Myzus persicae*. Larval and pupal development and survival of all species and reproductive parameters of *H. axyridis* were similar to published results. The system provides a suitable tritrophic exposure route, enables *ex-ante* evaluation of stressors, and improves the accuracy of the assessment.

Key words: beneficial insects, biosafety, ecotoxicology, exposure routes.

INTRODUCTION

Insect non-target predators can be exposed to environmental stressors, such as *Bt* entomotoxins in genetically modified (GM) plants, directly by consumption of parts of the GM plant or indirectly by predation on herbivores that themselves had fed on a GM plant (Groot and Dicke 2002, Romeis et al. 2006, Lövei et al. 2009). An ecotoxicological evaluation of effects on predators is often made by: a) exposing them to GM plant parts (such as pollen) or to an herbivore that fed on the GM plant, or b) by inoculating (or spraying) an environmental stressor on artificial diets or on predator food. In the first case, the environmental risk assessment is constrained to occur after the development of the technological product (e.g. GM plant) containing the environmental stressor, and in the second case, by the power of the ecological inferences that can be made using artificial exposure routes that do not fully simulate nature exposure. In this work we present a practical system that can be used to make an *ex-ante* ecotoxicological analysis of environmental stressors on aphidophagous predators through an ecologically relevant tritrophic exposure. We evaluate its practicality using three common generalist predator species that feed on pest aphid species.

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MATERIALS AND METHODS

Preparation of the System

The system (Douglas and van Emden, 2007) was made with transparent acrylic tubes (2.5 cm diameter x 2.5 cm height, wall thickness 0.35 cm) with two layers of parafilm M on one end forming a sachet, inside of which was 150 to 500 µl of liquid diet for rearing aphids (Dadd and Mittler 1966). Each tube required two square pieces of parafilm (Fig. 1a). One parafilm piece was stretched in both directions and attached to one end of the tube (Fig. 1b). The tube with the parafilm attached and the second piece of parafilm were sterilized by UV

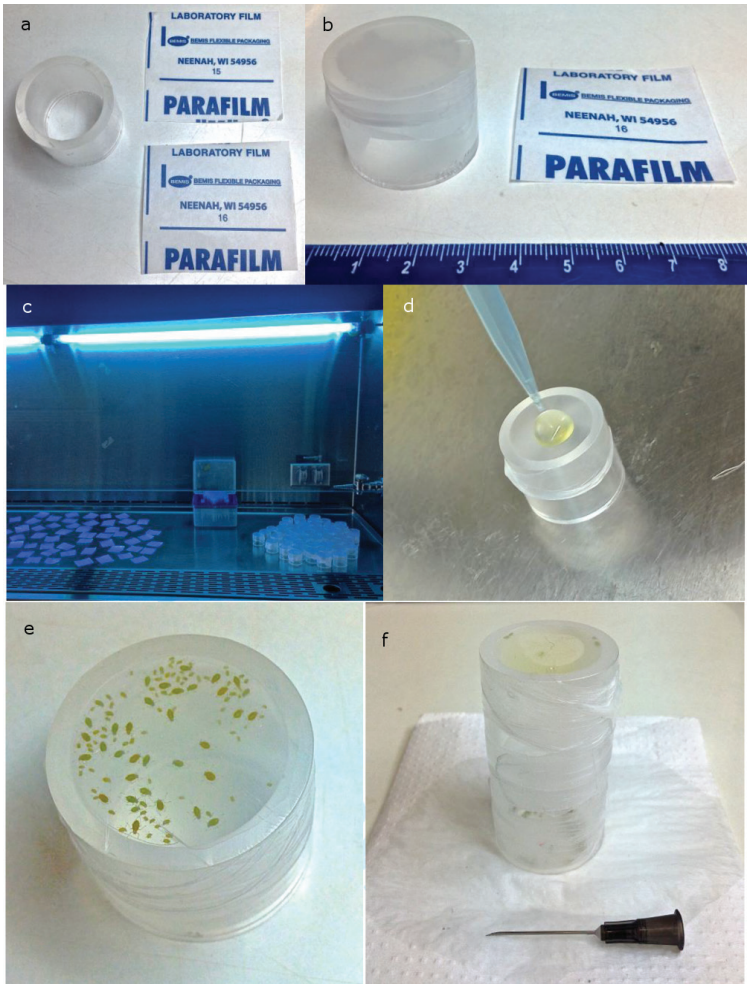

Figure 1 - Artificial tritrophic system for rearing aphids and aphidophagous predators: **a)** Tube and two Parafilm M pieces; **b)** Tube with one parafilm layer attached and a second parafilm piece, ruler with scale in cm; **c)** Sterilization of the system components by UV irradiation in a laminar flow hood; **d)** Pipetting of the diet onto the parafilm layer attached to the tube; e) Completed artificial aphid feeding system containing M. persicae feeding from the lower parafilm layer inside the tube; **f)** Aphid transfer to a tube with new diet. First the old and new tube are coupled together using a parafilm strip, then the diet is drained from the old tube by making holes with a needle and putting it upside down on absorbent paper. The aphids move from the old tube to the new one.

radiation for 30 to 40 min in a laminar flow hood (Fig. 1c). The liquid diet was sterilized by filtration using a filter (pore size of 0.22 μm) coupled to a plastic syringe in a laminar flow hood. The feeding tubes were finished inside a laminar flow hood by pipetting (with sterilized tips) the filtered diet on the sterilized layer of parafilm attached to the tube (Fig. 1d), and covering it with the sterilized face of the second parafilm piece, after stretching it in both directions. When the tubes were finished, *M. persicae* aphids were carefully transferred into them using a paint-brush (#2).

Rearing Predators in the Artificial Tritrophic System

Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), and the predaceous ladybird beetles, *Cycloneda sanguinea* and *Harmonia axyridis* (Coleoptera: Coccinellidae), and green lacewing, *Chrysoperla externa* (Neuroptera: Chrysopidae), were obtained from laboratory colonies and reared in growth chambers at 25±2°C and 16:8 LD, based on Paula et al. (2015) and Carvalho and Souza (2009). Unfed, recently emerged (<24 h) coccinellid and chrysopid neonates were individually (10 replicates each species) transferred to the system containing *M. persicae* aphids that had fed on the diet for at least 24 h and reared in a growth chamber at 25±2°C and 16:8 LD. The predator larvae were transferred daily to new tubes containing aphids feeding on the diet. Development time and survival (larval and pupal) were measured. Recently emerged adults (<24 h) were individually weighed and sexed. In addition, unfed recently (<24 h) emerged *H. axyridis* couples ($n \geq 10$) were individually transferred to the system containing 100 *M. persicae* aphids and reared for 20 days, as mentioned previously. The number of eggs laid and egg development time were recorded. Comparisons between our experimental values and published values were made using Welch's *t*-test (assuming unequal sample size and unequal variance) with a Bonferroni correction for the *P-value* when there were multiple comparisons.

RESULTS AND DISCUSSION

Overall the system provided suitable conditions for rearing the predator species (Tables I to III). Although we noted many differences in the biological parameter values compared to published values, there were no consistent differences, indicating that the performance of the predators in the system was within the normal range of variation of other rearing systems. Therefore, the system can be used in ecotoxicological tests as a tritrophic route of exposure for aphidophagous predators to several kinds of environmental stressors that can be added directly in the aphid diet, such as *Bt* proteins (Paula et al. 2015), dsRNAi and entomopathogenic agents (e.g., bacteria).

The main advantages of using this system are a) independence of the aphid from its host plant, enabling evaluation of an environmental stressor early in the development of a GM plant, and b) control of the exposure of the environmental stressor, in terms of concentration, homogeneity and constancy over time. In the first case, early ecotoxicological tests can provide a basis to optimize an intended technology (e.g., to produce an entomotoxin that is more species-specific) or even support the decision making process of continuing investments in development or not. In the second case, the control of exposure of an environmental stressor has fundamental relevance to risk analysis as it provides higher accuracy and precision in the estimation of potential effects by reducing extraneous sources of variability within and across treatments, thereby reducing residual error caused by varied expression of the stressor in the GM plant in response to the environment conditions, tissue type, developmental stage, or difference in variety/cultivar (genetic background) (Hilbeck et al. 2006; Romeis et al. 2008).

TABLE I

Biological parameter values (average ± SD or SE) for *Cycloneda sanguinea* immatures reared in the artificial tritrophic system and compared with the published literature. Parentheses are number individuals tested, and *P* is the Bonferroni *P*-value that the literature value differs from the artificial tritrophic system. The parameters that differed from the artificial tritrophic system are in bold when the published value was better and underlined when worse.

	Artificial tritrophic system	Santa-Cecília et al. (2001)	Isikber and Copland (2002)	Funichello (2010)	Souza et al. (2013)
Experimental conditions					
Temperature (°C), R.H. (%), photophase (h)	25±2, nr, 16	25±2, 70±10, 12	22,5±1, 60±5, 16	25±1, 70±5, 12	25±2, 60±10, 12
Prey	*M. persicae*	*Schizaphis graminum*	*M. persicae*	*A. gossypii*	*A. gossypii*
Larvae					
Development time (d)	9.33±0.12[2] (56)	8.35±0.32[2] (8)	**8.1±0.2[1] (10)**	10.02±0.36[2] (40)	10.58±1.99[1] (40)
P		0.083	**6.24×10^{-12}**	0.232	0.002
Survival rate (%)	73.42±4.97[2] (72)	80 (16)	77 (13)	**95 (40)**	**100 (40)**
P		0.944	0.998	**0.010**	**4.10×10^{-4}**
Pupae					
Weight (mg)	13.38±0.56[2] (55)	15.0±1.48[2] (16)	-	-	-
P		0.319	-	-	-

nr: not recorded; [1]SD; [2]SE.

TABLE II

Biological parameter values (average ± SD or SE) for *Harmonia axyridis* reared in the artificial tritrophic system and compared with the published literature. Parentheses are number of individuals tested, and *P* is the Bonferroni *P*-value that the literature value differs from the artificial tritrophic system. The parameters that differed from the artificial tritrophic system are in bold when the published value was better and underlined when worse.

	Artificial tritrophic system	Lamana and Miller (1998)	Stathas et al. (2001)	Matos and Obrycki (2006)	Chen et al. (2012)
Experimental conditions					
Temperature (°C), R.H. (%), photophase (h)	25±2, nr, 16	26±2, 50-70, 16	25±1, 65±1, 16	24±1, nr,16	25±0,5, 66±5, 16
Prey	*M. persicae*	*Acyrthosiphon pisum*	*A. fabae*	*M. lythri*	*Chaitophorus populeti*
Larvae					
Development time (days)	11.20±0.12[2] (103)	**10.2±1.0[1] (142)**	-	13.3±0.2[2] (24)	**9.3±0.58[1] (30)**
P		**3.29x10^{-10}**	-	8.72x10^{-11}	**0**
Survival rate (%)	73.81±3.92[2] (126)	86.2 (142)	88.7 (30)	-	90 (30)
P		0.050	0.395	-	0.158
Pupae					
Development time (d)	4.59±0.17[2] (27)	4.5±0.3[1] (142)	-	4.6±0.1[2] (24)	5.1 ± 0.46[1] (30)
P		0.918	-	1	0.036
Survival rate (%)	82.35±6.54[2] (34)	-	100 (30)	-	-
P		-	0.022	-	-
Weight (mg)	23.87±0.55[2] (68)	-	-	-	-
Larval to adulthood					
Development time (d)	14.36±0.16[2] (28)	-	-	17.8±0.2[2] (24)	-
P		-	-	1.45x10^{-16}	-
Adults					
Female weight (mg)	23.43±0.64[2] (14)	-	-	-	**27.0 ± 2.24[1] (10)**
P		-	-	-	**0.001**
Male weight (mg)	20.61±0.73[2] (18)	-	-	-	**24.8 ± 1.47[1] (10)**
P		-	-	-	**5.45x10^{-5}**
Sex ratio (% of females)	39.29±9.23[2] (28)	-	-	-	55.0
Reproduction					
Preoviposition period (d)	6.05±0.33[2] (20)	-	7.2±1.12[1] (30)	-	7.4 ± 1.74[1] (10)
P		-	0	-	1.63x10^{-08}
Daily fecundity (eggs per female)	40.59±4.32[2] (20)	-	-	-	24.4 ± 4.86[1] (10)
P		-	-	-	0.002
Eclosion rate (%)	43.43±6.88[2] (20)	-	-	-	-
Development time (d)	2.34±0.16[2] (20)	2.8± 0.30[1] (142)	-	-	-
P		0.010	-	-	-

nr: not recorded; [1]SD; [2]SE.

TABLE III

Biological parameter values (average ± SD or SE) for *Chrysoperla externa* immatures reared in the artificial tritrophic system and compared with the published literature. Parentheses are number of individuals tested, and *P* is the Bonferroni *P*-value that the literature value differs from the artificial tritrophic system. The parameters that differed from the artificial tritrophic system are in bold when the published value was better and underlined when worse.

	Artificial tritrophic system	Barbosa et al. (2006)	Murata et al. (2006)	Lira and Batista (2006)	Schlick-Souza et al. (2011)
Experimental conditions					
Temperature (°C), R.H. (%), photophase (h)	25±2, nr, 16	25±1, 70±10, 12	25±2, 75±10, 14	25±5, 80±5, 12	25±2, 60±10, 10
Prey	*M. persicae*	*M. persicae*	*Anagasta kuehniella*	*Hyadaphis foeniculum*	*A. gossypii*
Larvae					
Development time (d)	9.90±0.16[2] (30)	10.6±0.11[2] (37)	**9.02± 0.09[2] (30)**	8.6±0.4[2] (10)	9.59±0.65[1] (39)
P		0.003	**7.29x10^{-05}**	0.052	0.374
Survival rate (%)	75.00±6.85[2] (40)	92 (37)	**96.67 (30)**	-	81.25 (48)
P		0.122	**0.022**	-	-
Pupae					
Development time (d)	9.79±0.22[2] (24)	9.7±0.09[2] (36)	-	12.60±0.43[2] (6)	12.33±0.49[2] (31)
P		0.966	-	0.002	8.50x10^{-05}
Survival rate (%)	80.00±7.30[2] (30)	97 (36)	86.21 (29)	-	81.14 (39)
P		0.057	0.892	-	0.995
Larval to adulthood					
Development time (d)	19.71±0.21[2] (24)	-	-	20.25±0.94[2] (6)	-
P		-	-	0.593	-
Adults					
Female weight (mg)	4.67±1.20[2] (8)	-	-	-	-
Male weight (mg)	5.54±0.76[2] (16)	-	-	-	-
Sex ratio (% of females)	33.3±9.62[2] (24)	-	44.0 (25)	66.7 (6)	-
P		-	0.690	0.259	-

nr: not recorded; [1]SD; [2]SE.

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