The use of various oviposition structures for the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomydae) in improving the reproductive process in captivity

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Abstract. The research in this paper is aimed at determining the optimal structure for *Hermetia illucens L.* female oviposition and the most efficient structure under an egg mass harvesting aspect, by appreciating the preferences of *H. illucens* for a certain type of material. Four experimental cages were designed, in which identical microclimate conditions were provided and the same type of organic substrate with an attractant function for oviposition - brewers grains - was used. The difference consisted in the oviposition structure, which was composed of different material for each experimental cage. To this purpose, the following materials were used in each experimental cage: wood (WSEC), glass (GSEC), corrugated cardboard (CSEC) and plastic material (PSEC). 50 g of pupae were counted, measured and weighed for each experimental cage. Once the reproduction process was completed, the adult hatching rate was determined, the laid egg masses were counted, and their distribution was recorded according to the utilized material. Adult hatching percentages of over 98% were obtained in all four experimental cages. The largest number of egg masses (77) was recorded for WSEC, followed by CSEC (76), GSEC (70) and PSEC (52). Throughout the breeding period, most of the egg-masses were oviposited on natural materials. In conclusion, wood and corrugated cardboard are materials that are best suited to be used in designing the oviposition and mass-collecting structure for the black soldier fly, wood being recommended for a better efficiency in mass-collecting.

Key Words: hatching rate, reproduction, organic substrate, pupae, egg mass.

Introduction. Economic, nutritional and environmental advantages make insects an attractive protein source (Cullere et al 2016; Józefiak et al 2016; Tran et al 2015), Insects can be reared in small spaces (Rumpold & Schlüter 2013) without requiring many resources (FAO 2013). Insects can be fed organic wastes (Gabler 2014; Makkar et al 2014) which are thus transformed into high-value protein biomass (De Marco et al 2015; van Huis 2013). Therefore, the waste amount is reduced (Newton et al 2005 a) and valuable protein feed that can be used in monogastric animal farming is obtained (Sánchez-Muros et al 2014; Veldkamp et al 2012). Insects develop and breed easily (Cortes Ortiz et al 2016), resulting in a large amount of larval biomass over a short time period (Henry et al 2015; Feedipedia 2012).

Originating from the southern hemisphere, namely the tropical, subtropical and warm temperate areas of South America, the black soldier fly (*Hermetia illucens* Linnaeus, 1758) is part of the Diptera order, Stratiomyidae family (Makkar et al 2014; Park et al 2016; Tomberlin et al 2002; Surendra et al 2016). It colonizes organic waste (Nguyen et al 2015; Meneguz et al 2018) and it can often be found in the vicinity of livestock farms, where larvae feed on the resulting manure or various vegetal or household wastes (Newton et al 2005 a, b; Wang & Shelomi 2017). It is not attracted by human food or habitat (Furman et al 1959; Park 2015) and it contributes to housefly (*Musca domestica*) population reduction (Myers et al 2008), limiting possible infection outbreaks or disease transmission (Shepperd et al 1994; Tomberlin et al 2002).

Holometabolic Insect (Caruso et al 2013), after complete transformation, *H. illucens* larvae and adults exhibit different morphological and behavioral characteristics. The oral apparatus and digestive tract of the adult does not develop, and the adult does not feed, surviving from reserves accumulated during the larval period (Newton et al 2005 a, b; Tomberlin et al 2002). Larvae can consume considerable amounts of decomposing organic material (Cortes-Ortiz et al 2016; Dortmans et al 2017; Holmes et al 2016). At the end of the larval period, they cease to consume the substrate, the tegument is keratinized (Oliviera et al 2015; Park 2015) and begin to migrate, looking for a dry and protected place for pupation (Barros-Cordeiro et al 2014). The duration of the pupae stage is very variable (from 8-14 days to 5 months) depending on the environmental conditions (Tomberlin et al 2002; Zhang et al 2010).

After hatching, the flies are mating within 2-4 days and the oviposition takes place in the first two days after mating (Tomberlin & Sheppard 2001). The female flies, attracted by the odor generated by the decomposing organic matter (Tomberlin et al 2002), lay their eggs in dry (Booth & Sheppard 1984; Tomberlin et al 2009) and sheltered nearby crevasses (Hardouin & Mahoux 2003; Diener et al 2011). Eggs of very small size (1-2 μg) are deposited in masses and, once oviposited, the hatching takes place in about four days (Tomberlin & Sheppard 2001; Zhang et al 2010). Shortly after ovipositing, the adults die due to the depletion of energy reserves fully channeled to the breeding process (Sheppard et al 1994; Tomberlin et al 2002).

Black soldier fly rearing systems assume development and reproduction optimal artificial conditions. Productive and reproductive performance is influenced by physiological factors, as well as by environmental and technological parameters. Development and life cycle are dependent on the existence of optimal temperature, humidity and luminosity factors, but also on larvae feeding and development substrates (Oliveira et al 2015; Diener et al 2011; Hardouin & Mahoux 2003). Insects can be reared in mesh cages or in plastic containers of varying sizes (Nakamura et al 2016; Sheppard et al 2002). These systems include artificial plants, light sources, larvae feeding substrates and reproduction structures (Park 2015; Chia et al 2018; Dortmans et al 2017; Sheppard et al 1994).

A better control of oviposition and mass harvesting can significantly increase egg production and, implicitly, larvae production that can be used for recycling organic matter (Caruso et al 2013; Holmes et al 2013). In order to improve the breeding process, in addition to ensuring environmental conditions, it is necessary to use a structure that allows efficient oviposition and mass collection.

The aim of this study was to test different materials and to determine which material is best suited in designing the oviposition structure, black soldier fly reproduction being a life cycle stage that still imposes some issues in rearing the species in captivity.

Material and Method. The experiments were carried out in the laboratory of Ecology and Environmental Protection of the Animal Science and Biotechnologies Faculty, UASVM Cluj-Napoca, in April-May 2018. By the conducted activities, the objective was to determine the preferences of the black soldier fly for a certain type of material and structure for oviposition. The biological material used originates from our own source. Depending on material and structure used, the number of masses was determined and the uniformly distribution and grouping of the masses were observed and recorded.

Structure designing for oviposition and mass collection. Four types of different materials were used, as shown below:

- wood structure (WS): wooden plates overlapping 3 mm by means of some wooden spacers attached inside (Figure 1 WS)
- glass structure (GS): glass plates overlapping 3 mm by means of some wooden spacers attached inside (Figure 1 GS)
- corrugated cardboard structure (CS): corrugated cardboard overlapped plates which laterally present openings from its specific corrugation construction (Figure 1 CS)

- plastic structure (PS): two plastic spheres which present overlapping ridges, with a distance among them of 5 mm (Figure 1 PS).

In order to assure the stability of the structures and their suspension above the organic substrate, wood support plates propped on the longitudinal walls of the box with the organic material were used for the first three types of experimental structures. The plastic structures were propped by a rod of the same material, similarly positioned (Figure 1).

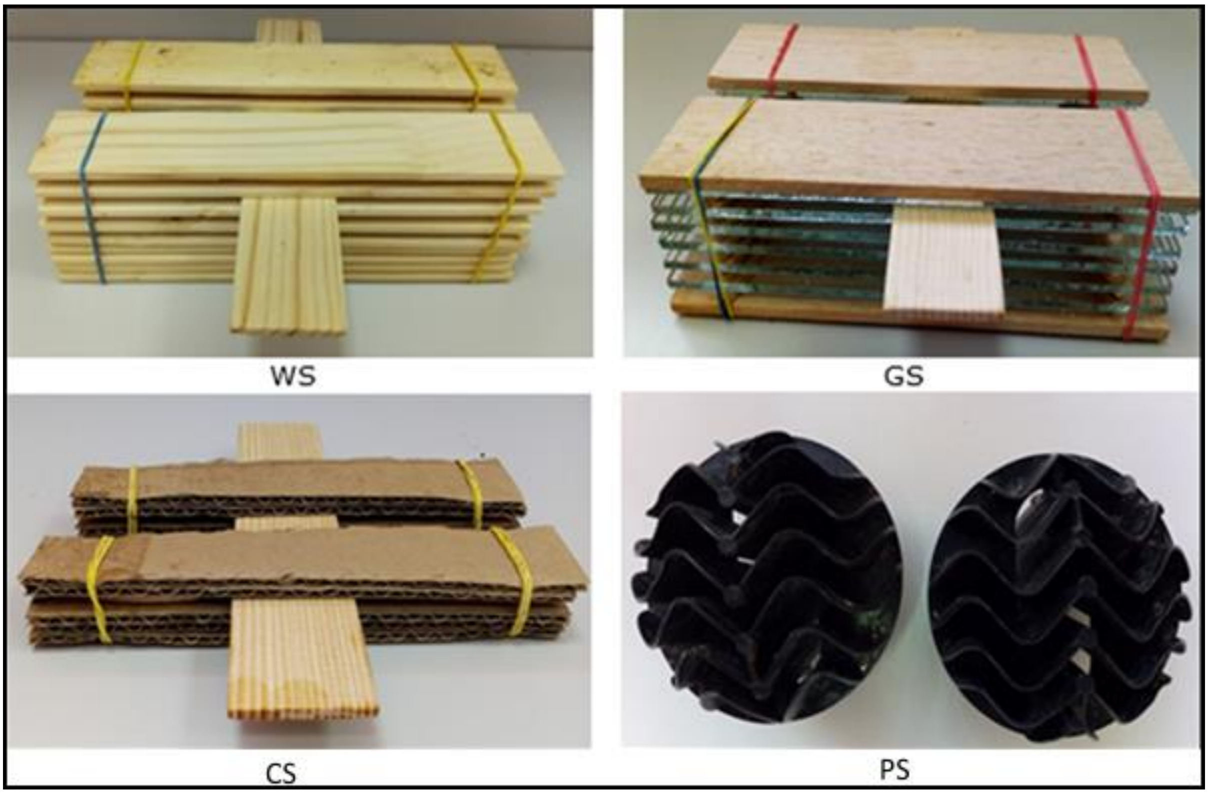

Figure 1. Designing and preparing the oviposition structures (original).

Managing the experimental design. To carry out the reproduction activity, the pupae were placed in separate experimental cages, with the organic substrate and oviposition structure (Figure 2 A).

Figure 2. Experimental cage (A) and box with substrate (B) (original).

In each experimental cage, the same microclimate conditions were ensured: 22-28°C temperature, 40-60% humidity, 8 hours/day artificial light. The biological peculiarity of the species for dry and dark places, favorable for oviposition, but also the existence of sufficient humidity has been taken into account. For this purpose, black plastic boxes (3L) were used, in which barley brewer grain (0.6 Kg) was introduced to stimulate the reproductive act. Each type of structure for oviposition was placed into such a box, over which a plastic lid was applied, leaving enough space for the females attracted to the organic material to enter for oviposition (Figure 2 B).

Measurements and determinations. In each experimental cage, 50 g of pupae were placed, numbered, measured (with a Stainless Hardened electronic caliper) and weighed (with a Pro Explorer analytical balance, 110 g capacity and 0.1 mg precision). Individual values for pupae weight and length were statistically processed (Microsoft Office Excel) and mean and standard deviation were displayed (Mean±SD). After the reproductive activity was completed, the hatching rate was calculated (by a ratio of hatched adults to the total number of pupae), the number of masses was determined for each experimental cage and the mass distribution was recorded for each type of material used.

Results and Discussion. The average pupae size of 0.16 g weight and 1.95 cm length reveals values that correspond to those reported by other authors (Tomberlin et al 2002; Newton et al 2005), and, in terms of numbers, the values were very similar for each experimental space (Table 1).

The first adult specimens hatched after 3 days and the process continued until day 5. The average hatching percentage of the flies was 98.91%. Of the 1749 used pupae (200 g), 1730 adults hatched. In each experimental cage, the hatching percentage recorded very close values (Table 1). From the day after hatching, the occurrence of the first mating processes could be observed, further taking place for 6 days. The ovipositioning process was observed 1-2 days after the start of mating and took place for 4 days. After finishing the mating process, oviposited masses were counted for each experimental cage and their distribution on each structure and material was recorded.

Table 1

Average pupae size, distribution in the experimental space and hatching absolute and relative values

Experimental cage	*Pupae quantity (g)*	*Number of pupae (pieces)*	*Pupa weight (g)*	*Pupa length (cm)*	*No. of hatched adults (pieces)*	*Hatching ratio (%)*
			Mean±SD	*Mean±SD*		
WSEC	50	442	0.16±0.28	1.95±0.22	437	98.86
GSEC	50	456			452	99.12
CSEC	50	428			420	98.13
PSEC	50	423			421	99.52

WSEC - wood structure experimental cage; GSEC - glass structure experimental cage; CSEC - corrugated cardboard structure experimental cage; PSEC - plastic structure experimental cage.

The varied distribution of pupae (Table 2) on the different types of materials present in the experimental cages (wood, corrugated cardboard, glass plastic and organic material) highlights the ovipositioning preference of the females for a certain category of material.

Concerning the wooden structure experimental cage (Figure 3), the structure concentrates 93.50% of the total number of masses ovisposited in the experimental cage. This aspect can be considered advantageous because the egg harvesting from the smooth surface of the wood is accessible and the structure can be reused.

Likewise, the corrugated cardboard structure (Figure 3) proved to be a material preferred by female black soldier flies, accumulating 80.26% of the total number of masses.

Table 2

Mass number and distribution in each experimental cage, depending on the oviposition structure

	Oviposition site	*Number*	*%*
WSEC	wooden structure	72	93.50
	plastic lid	3	3.90
	contact area of plastic box and organic material	2	2.60
	Total	**77**	100
GSEC	wooden spacers for glass plates	34	48.57
	plastic lid	11	15.71
	glass-wood contact surface	5	7.15
	glass plates	2	2.86
	contact area of plastic box and organic material	18	25.71
	Total	**70**	100.00
CSEC	corrugated cardboard structure	61	80.26
	plastic lid	1	1.31
	contact area of plastic box and organic material	11	14.47
	organic substrate	3	3.96
	Total	**76**	100.00
PSEC	inside the plastic bio ball	8	15.39
	plastic lid	27	51.92
	contact area of plastic box and organic material	17	32.69
	Total	**52**	100.00

WSEC - wood structure experimental cage; GSEC - glass structure experimental cage; CSEC - corrugated cardboard structure experimental cage; PSEC - plastic structure experimental cage.

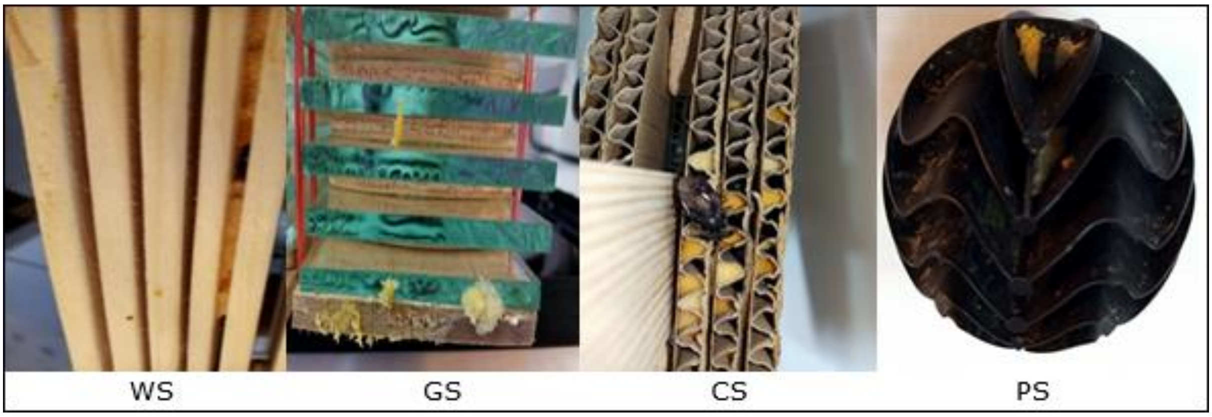

Figure 3. Masses oviposited in each type of structure (original).

The glass structure (Figure 3) was the least preferred (2.85%), most of the imago females pursuing the wooden spacers distancing the glass plates (48.57%), the plastic area of the box in contact with organic material (25.71%) and the plastic lid (15.71%). The wooden surface was limited, thus determining the oviposition on adjacent materials.

In the PSEC, only 15.38% of the total number of masses was oviposited inside the plastic spheres (Figure 3). Most masses (27), almost 52%, could be observed on the interior side of the plastic lid of the substrate box. Furthermore, 17 masses, representing 32.69%, had been found at the plastic walls and organic material contact areas. In the absence of an optimal oviposition structure, a clear spreading of the oviposition sites is noted. The female effort of searching and identifying suitable oviposition sites is highlighted by the diversification of the oviposition sites, fact confirmed by the small number of masses (52) recorded in this experimental cage. These results disagree with the assertions made by Dortmans et al (2017), recommending the use of plastic bio balls for efficient oviposition and egg harvesting, as wood or cardboard structures absorb moisture. However, most research published recommends cardboard or wooden structures due to adequate mass harvesting results (Tomberlin & Sheppard 2002; Nguyen et al 2013; Holmes et al 2016). Nonetheless, it should be stressed that the cardboard structure does not allow the manual collection of the eggs or the reuse of the support but provides additional protection of the masses due to its specific corrugated structure.

Under these conditions, it can be stated that wood and corrugated cardboard (obtained from wood processing), as natural materials, represent an optimal structure for mass laying, preferred by female flies because of their close resemblance to the natural habitat of the species. A number of 133 masses were laid on wood and cardboard (93.01%), while only 10 masses were deposited on the glass and plastic structures (6.99%), from the total number of masses recorded on the studied oviposition structures. This distribution shows the preference of the black soldier fly to oviposit on natural materials.

According to the recorded data (Table 2), there are no differences between the first 3 experimental cages (WSEC, GSEC, CSEC) in terms of the total number of oviposited masses. An explanation may consist in the fact that oviposition structures made of natural materials that, according to obtained results, stimulate the reproductive process, were placed inside the first three cages, while in PSEC the structure was made of synthetic materials. On the other hand, it can be said that the most orderly distribution of the masses appears in the WSEC. For this cage, the smallest number of differentially distributed masses was recorded, 3 on the plastic lid and 2 inside the box, in contact with the organic substrate. As far as the GSEC and CSEC are concerned, even if the same approximate number of masses was recorded, they presented a heterogenous distribution, in both cases a considerable number of masses being distributed in the organic substrate contact area. The disorderly distribution of masses is undesirable because the egg production cannot be efficiently controlled, which is essential for mass production of *H. illucens*, as Pastor et al (2015) mention.

The obtained results confirm and show that the black soldier fly imago is attracted to natural materials (wood and cardboard) even if there are also synthetic materials close by. Although plastic material was present in each experimental cage, either in form of a container or in the form of an oviposition structure and had a greater exposed surface than the materials of natural origin, the difference in the number of masses oviposited is notably in favor of the natural components (Figure 4). Considering this, if we strictly refer to the experimental cages in which both natural and synthetic materials were present, for the fairness of the study, the experimental cages that fulfill this condition are WSEC, GSEC and CSEC, because in PSEC only plastic material was present (the bio balls and the box). Hence, these results in a total of 223 masses, of which about 78% were oviposited on natural materials and more than 21% were oviposited on synthetic components (Figure 4).

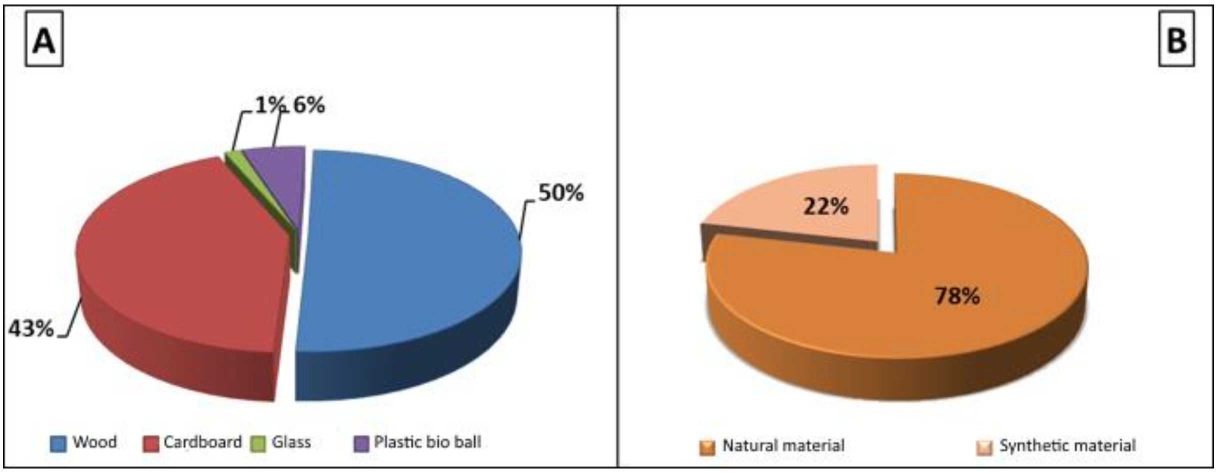

Figure 4. Mass distribution according to the oviposition structure (A) and the preference for material type (B).

Conclusions. The most suitable materials for oviposition in *H. illucens* are wood and cardboard. Even if the glass has the advantage of a more efficient egg harvesting and reuse after disinfection, the very small number of masses obtained does not recommend it for designing the oviposition structure. Plastic material can serve as an oviposition structure only in the absence of natural materials. By ensuring specific environmental conditions, the breeding activity can be controlled to obtain a considerable number of eggs for larvae production. The use of barley brewer grain as an oviposition substrate has been shown to be a good stimulator of the reproductive function. The reproductive process can be controlled and intensified to obtain a noteworthy number of larvae and prepupae to be used for various purposes.

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