A Behavioral Method for Separation of House Fly (Diptera: Muscidae) Larvae From Processed Pig Manure

HELENA ČIČKOVÁ,[1,2,3] MILAN KOZÁNEK,[1] IVAN MORÁVEK,[4] AND PETER TAKÁČ[1,2]

J. Econ. Entomol. 105(1): 62–66 (2012); DOI: http://dx.doi.org/10.1603/EC11202

ABSTRACT A behavioral method applicable in biodegradation facilities for separation of house fly (*Musca domestica* L.) larvae from processed pig manure is presented. The method is based on placing a cover over the larval rearing tray, while escaping larvae are collected in collection trays. Separation units must be placed in a dark room to avoid negative phototactic responses of the larvae. After 24 h of separation, over 70% of the larvae escaped from processed manure and were collected in collection trays. Most of the larvae pupated within 48 h after separation. Mean weight of pupae recovered from manure residue was not significantly different from mean weight of pupae of separated individuals. Eclosion rate of pupae recovered from manure residue was significantly lower than eclosion of separated individuals, and was strongly related to separation success. Factors responsible for escape behavior of larvae are discussed.

KEY WORDS escape behavior, hypoxia, pig manure, separation, biodegradation

Processing animal manure using house fly larvae is an approach that can substantially reduce manure water and organic nitrogen content (Barnard et al. 1998, Beard and Sands 1973, Golubeva and Erofeeva 1981), and reduce problems with manure storage, such as the development of fly larvae. With processed manure not being suitable for fly development (Barnard et al. 1998, Yang et al. 2004), processed manure can be a well-balanced organic fertilizer (Kováčik et al. 2010). Reared house fly larvae or pupae can be used as a valuable feed supplement for fish or poultry (e.g., Fasakin et al. 2003, Hwangbo et al. 2009, Ogunji et al. 2008, Zuidhof et al. 2003) or for rearing of specific fly parasitoids as natural biocontrol agents (Floate 2002, Kaufman and Geden 2009). Both products of biodegradation can be of economic interest for farmers.

An effective and reliable method to separate house fly larvae or pupae from the breeding medium is required to obtain high quality final products. Difficulties in harvesting the larvae from processed organic waste may limit their potential use (Yaqub 1997), and decrease economic benefits and attractiveness of biodegradation to the farmers.

The most effective separation methods known to date are small-scale laboratory extraction methods for larvae (e.g., Barnard 1995, Tobin and Pitts 1999). The flotation method can be very reliable in recovering the larvae (Tobin and Pitts 1999) and pupae, but during their collection, some manure particles float on the water solution making it difficult to separate insects from the substrate. Moreover, the manure residue turns into an undesirable aqueous waste product. Sieving can separate particles smaller and larger than pupae, and two final products of relatively good quality can be obtained; however, the sifted material containing manure particles that match the size of pupae can cause difficulties when using the insects. It also requires the substrate with pupae to be relatively homogeneous and dry. This is not always possible to achieve without using extra procedures.

Separation methods based on behavioral responses of the larvae to specific stimuli seem to be more promising. Because it is the larvae that escape from the substrate (manure), the harvested biomass contains very few impurities. Various stimuli can be used to elicit escape behavior of the larvae including heat, light, and excessive moisture. These methods, however, have not been tested as intensively as physical methods of separation.

Eby and Dendy (1976) used intensive light to separate 75–90% of the larvae using an industrial screen separator. Similar results were achieved with a device for rearing and separation of the larvae that allows hermetic enclosure of the container with developing insects. This method is presumably based on increasing concentration of metabolic products (CO_2, NH_3, H_2O) and hypoxia, and results in 71–96% separation success (Sorokoletov 2006). While this method benefits from using less energy and low maintenance costs, a notable disadvantage of the whole system is extremely high (78%) mortality of the larvae before the separation (Sorokoletov 2006).

[1] Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia.
[2] Scientica, s.r.o., Hybešova 33, 831 06 Bratislava, Slovakia.
[3] Corresponding author, e-mail: helena.cickova@savba.sk.
[4] Institute of Manufacturing Systems, Environmental Technology, and Quality Management, Slovak University of Technology, Námestie slobody 17, 812 43 Bratislava, Slovakia.

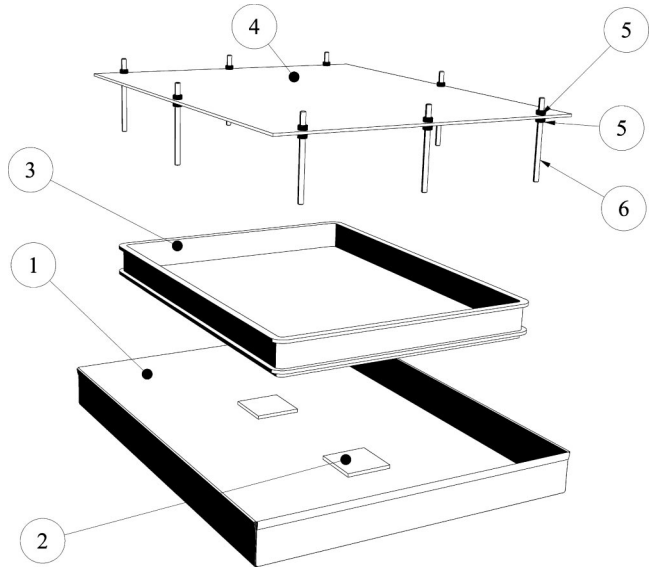

Fig. 1. Collection unit. 1 – metal collection tray, 2 – plastic stand, 3 – larval rearing tray, 4 – cover, 5 – nuts, 6 – bolt. Substrate with larvae is not shown.

In the current study we evaluate the separation method based on escape behavior of larvae exposed to lack of fresh air for extraction of house fly larvae from processed pig manure in settings that allow reasonable survival rate of the larvae during the rearing process. This technique seems to be very promising because of energy savings and easy performation.

Materials and Methods

The house fly strain used in all experiments was colonized in 2005 from wild flies caught near a pig farm in Miloslavov, Slovakia, as described earlier (Pastor et al. 2011). Adults were kept in 30 × 30 × 30 cm screen cages at 25 ± 2°C, 40–60% RH, and a photoperiod of 12:12 (L:D) h and provided with an unlimited access to water and a mixture of powdered milk and sugar (1:1). Eggs were collected with an oviposition device, which consisted of a 200 ml cup containing 50–100 ml of water. The lid of this cup had a narrow longitudinal opening that allowed insertion of a sponge strip. A bag containing 50 ml of pig manure wrapped in 15 × 15 cm black cloth and closed with a rubber band was placed on the lid so that the sponge would moisten the manure. A 500 ml cup with two 2 × 1 cm openings near its edges was inverted over the whole complex. The openings in the upper cup allowed the flies to enter the oviposition device. The flies were allowed to oviposit for 12 h. Fresh manure was seeded with house fly eggs (2 ml (≈22,000) eggs/5 kg manure) and larval rearing trays were kept at 24 ± 2°C, 40–80% RH, and a photoperiod of 12:12 (L:D) h. Pig manure used for rearing of the house fly larvae in all experiments was collected directly from the pens and contained up to 50% of sawdust, which was used as bedding for the pigs and could not be separated from the manure before use.

Separation Unit. The separation unit used for larval extraction from processed manure consisted of a metal collection tray (55 × 69 × 7 cm; 1 mm thick), two plastic stands (one or 2 cm high), a larval rearing tray (inner dimensions 37 × 47 × 7 cm) placed on the stands in the collection tray, and a plastic cover (4 mm thick, 43 cm wide, 63 cm long) held by eight bolts (12.5 cm long, 7 mm thick) around its edges that was placed over the larval tray (Fig. 1). When assembled, the bolts were located between the larval and collection tray and the nuts allowed individual adjustment of the height of the gap that formed between the cover and larval tray. The height of the gap was set to 3–4 mm. Separation units were placed into a trolley at least 50 cm above the ground (eight trays per trolley; Fig. 2) to avoid the negative effects of lower temperature on the larval development.

The Effect of Light on Separation Success. Four days after inoculation of eggs on the manure surface, the plastic larval rearing tray was placed into the collection tray and the separation unit was assembled (Fig. 1). Five separation units were placed into the trolley and kept in an illuminated separation room (24 ± 2°C, 40–60% RH, constant light). On the following day, five new separation units were assembled and placed into a dark separation room (24 ± 2°C, 40–60% RH, constant dark). The experiments were performed on two different days because of technical limitations of the facility (no two rooms in the biodegradation facility were identical in terms of environmental conditions and/or could be subjected to required light regime). The separation process was allowed to run for 24 h under both circumstances. Subsequently, larval and collection trays were taken off the collection units and transferred back to larval rearing room so that larvae remaining in both trays completed pupation. Separation success was calcu-

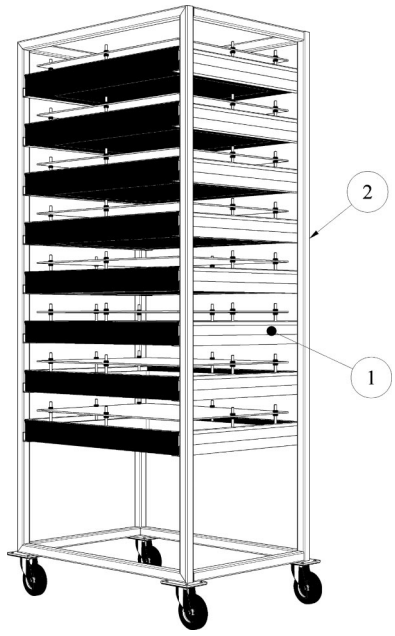

Fig. 2. Trolley with collection units. 1 – collection unit, 2 – trolley.

lated as the number of separated individuals (larvae and pupae) in the collection tray divided by the number of all larvae and pupae (separated individuals plus the pupae recovered from manure residue by flotation in water) obtained from every larval rearing tray.

Separation–Evaluation. After establishment of the negative effect of light on separation, a new experiment was set up to evaluate the effectiveness of separation under optimized conditions. A total of 24 replicates were evaluated after 24 h of separation in the dark separation room. After disassembling of separation units, larval and collection trays were transferred back to larval rearing room. After 48 h in larval rearing room, the number of pupae and larvae in each collection tray was counted and percentage of pupation was calculated. Five hundred pupae were counted and weighed (±0.0001 g) to determine mean weight of pupae. One hundred pupae were placed in a petri dish and kept at 25 ± 2°C and 40–60% RH to check eclosion rate. The manure with unseparated larvae was checked daily and once pupation finished (7–9th day since the inoculation of the eggs), pupae were separated by flotation in water and counted. Mean pupal weight and eclosion rate was checked as mentioned above. Separation success was calculated for every replication by dividing the number of separated individuals (larvae + pupae) by total (separated + unseparated) number of individuals.

Statistical Analysis. The effect of light regime on separation success as well as mean eclosions of separated and unseparated pupae were compared using Behrens–Fisher (t') test (Zar 2010). Mean pupal weight of separated and unseparated pupae was compared using a parametric t-test. Regression analysis was used to establish the relation between eclosion rate of unseparated pupae and separation success using a nonlinear regression analysis program NLREG v6.5 (Sherrod 2010). Descriptive statistics, t and t' tests were calculated with OpenOffice.org Calc according to the formulas proposed by Zar (2010).

Table 1. Influence of light on the separation of house fly larvae from pig manure (mean ± SEM)

Treatment	*N*	Separation success (%)
Light	5	3.73 ± 0.82a
Dark	5	69.17 ± 11.19b

Means in columns followed by different letters are significantly different ($P = 0.001$).

Results and Discussion

The Effect of Light on Separation Success. The repelling effect of light on larvae of numerous fly species is well known (Bolwig 1946, Strange 1961). Light had a strong influence on separation success: on average, only 3.7% of all house fly larvae were separated in the illuminated room, compared with 69.2% in the dark room ($t' = 5.812$, df = 4, $P < 0.001$; Table 1). Negative phototaxis of the larvae can be so strong that it was used as the main principle for removing the larvae from processed manure in the screen separator proposed by Eby and Dendy (1976).

Separation–Evaluation. Separation success after 24 h in the dark room reached 74.0 ± 4.7% (mean ± SEM). In eight cases the separation success was higher than 90%, and in three cases the separation success was <50%. Separated larvae were free of any manure particles.

The mechanism that forces the house fly larvae to escape from manure seems to be the lack of oxygen and accumulation of noxious metabolic products (Sorokoletov 2006). It was observed that the larvae of *Drosophila melanogaster* L. promptly exit their breeding medium and exhibit exploratory wandering behavior within seconds after the onset of oxygen deprivation (Wingrove and O'Farrell 1999). In agreement with this, the first house fly larva falling into the collection tray were observed within 1 h after assembling the separation units and placing them in the dark. This may be the time when rising levels of metabolic products in the medium began to be critical for the larvae. However, no experiments were carried out to determine the level of oxygen or other substances in manure during separation in the current study.

The efficiency of presented method in extracting the larvae from processed pig manure is lower than the method described by Sorokoletov (2006), who reached 84.0% separation success. This result is most likely caused by the different design of the device: Sorokoletov (2006) used an apparatus that could be hermetically enclosed and thus the amount of metabolic products responsible for the escape behavior of the larvae was higher. In our experiments, we used a

Table 2. Basic characteristics of separated and unseparated individuals after 24 h of separation from pig manure (mean ± SEM)

Individuals	*N*	Pupation after 48 h (%)	Mean wt of pupae (mg)	Mean eclosion (%)
Separated	24	88.22 ± 2.00	17.00 ± 0.63	92.83 ± 1.13a
Unseparated	24	—	18.23 ± 0.59	84.97 ± 3.62b

Means in columns followed by different letters are significantly different ($P = 0.05$).

system where a small gap allowed partial replenishment of fresh air and probably accounted for lower efficiency. The proportion of separated larvae is comparable with the results of Eby and Dendy (1976), who reported 75–90% separation success using their screen separator based on negative phototactic response of the larvae. The combination of light and heat in Tullgrene funnels is even more effective (close to 100%; Barnard 1995). However, the volume of samples that can be processed this way is limited and energy costs would also be high. It may not be suitable for large-scale rearing facilities. The behavioral method of separation of house fly larvae described in this paper is easily performed and does not require any complicated apparatuses or additional sources of energy.

Mean number of all individuals (separated + unseparated) recovered per larval tray was 11,030 ± 550. Overall egg-to-pupa survival varied from 27 to 70% with an average of 50.1%, which is higher than the average 22% survival reported by Sorokoletov (2006). This suggests that the larvae benefited from better environmental conditions during the rearing process before separation. Relatively high variation in larval survival was probably the consequence of variable nutritional value of the breeding substrate (manure with sawdust) because it was taken directly from the pig pens and was not homogenized before use. We must also note that the main objective in the biodegradation facility is to get well-processed manure residue. To achieve this, slightly excessive number of the eggs or maggots is applied to fresh manure. Competition for food among the larvae ensures that the highest possible amount of nutrients is recovered from the manure and stored in their bodies. These conditions necessarily result in increased larval mortality and decreased size of the maggots and pupae (Barnard et al. 1998, Moon et al. 2001).

Forty-eight hours after separation was complete, on average 88.2 ± 2.0% of separated larvae pupated. The mean weight of unseparated pupae was slightly, although not significantly, higher than that of separated pupae ($t = 1.434$, df = 46, $P > 0.05$; Table 2). This indicates that the larvae were separated from manure when they almost finished their natural feeding period.

Eclosion of adults was reduced for unseparated larvae compared with separated ones and the difference was significant ($t' = 2.076$; df = 27.440; $P < 0.05$), but the t' value was only little higher than critical value ($t_{0.05(2),27} = 2.052$). Closer examination revealed that the eclosion of unseparated pupae was related to separation success, with best fitting function being $Y = 95.663 - 2.797 * 10^{-26} * X^{13.612}$ (where Y is eclosion from unseparated pupae, and X is separation success; Fig. 3). This model is highly significant ($F = 22.25$; df = 2, 21; $P < 0.001$), and explains 67.93% of total variability in eclosion.

The decreased eclosion of adults from pupae recovered from the manure in the cases when separation success exceeded 70% suggests that there was a negative factor that was toxic and responsible for decreased eclosion of those individuals that stayed in manure during separation and were capable of pupation. It is probably the same factor responsible for escape behavior of the larvae, because eclosion of adults was closely related to separation success.

This study demonstrates high potential yields of the house fly larvae after biodegradation and separation process. However, because we did not determine the exact mechanism of the separation technique, further observations aimed to determine metabolic conditions and concentration of oxygen and other gases in manure during separation are needed. Additionally, the effect of gap size on the separation success and build-up of metabolic products within manure, should

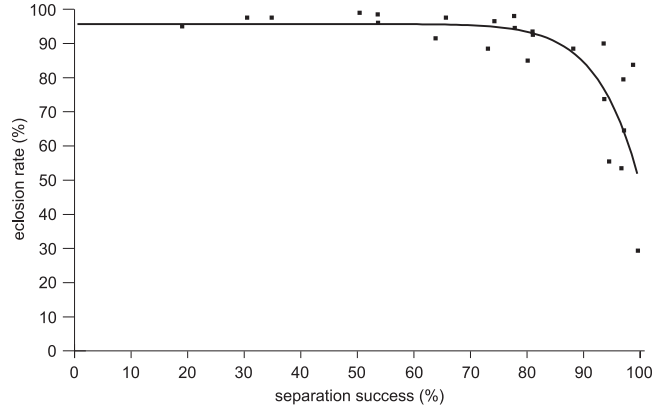

Fig. 3. Relationship between eclosion of unseparated individuals and separation success.

be examined to explain the factors responsible for the escape behavior of the larvae.

Development of an effective and reliable method of separation is a crucial step for functioning of a large-scale biodegradation facility. This study shows that the behavioral method for separation of the house fly larvae from processed pig manure is efficient, easy to perform and allows reasonable larval survival.

Acknowledgments

The authors are very thankful to Zdenka Kalaninová (FMFI, Comenius University) for her advice on regression analysis, and to Jerry Hogsette (USDA-ARS) and Tim Lysyk (Agriculture and Agri-Food, Canada) for reviewing and comments on the manuscript. This study was financially supported by Slovak Research and Development Agency, Grant No. VMSP-II-0025-09 The development and testing of the technology for the biodegradation of livestock manure.

References Cited

Barnard, D. R. 1995. Extraction of house fly (Diptera: Muscidae) larvae from poultry manure using Berlese/Tullgren funnels. Fla. Entomol. 78: 541–543.

Barnard, D. R., R. H. Harms, and D. R. Sloan. 1998. Biodegradation of poultry manure by house fly (Diptera: Muscidae). Environ. Entomol. 27: 600–605.

Beard, R. L., and D. C. Sands. 1973. Factors affecting degradation of poultry manure by flies. Environ. Entomol. 2: 801–806.

Bolwig, N. 1946. Senses and sense organ of the anterior end of the house-fly larvae. Videnskab. Medd. Dansk. Naturh. Foren. Kjobenhavn 109: 80–219.

Eby, H. J., and W. L. Dendy. 1976. An attempt to mechanize nutrient recovery from the animal waste by the use of house fly larvae. *In* Proceedings of Winter Meeting of American Society of Agricultural Engineers, technical paper, 14–17 December 1976.

Fasakin, E. A., A. M. Balogun, and O. O. Ajayi. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac. Res. 34: 733–738.

Floate, K. D. 2002. Production of filth fly parasitoids (Hymenoptera: Pteromalidae) on fresh and freeze-killed and stored house fly pupae. Biocontrol Sci. Tech. 12: 595–603.

Golubeva, E. G., and T. V. Erofeeva. 1981. On the use of house fly larvae for utilization of organic waste in biological human life support systems. Kosm. Biol. Aviak. Med. 15: 54–57.

Hwangbo, J., E. C. Hong, A. Jang, H. K. Kang, J. S. Oh, B. W. Kim, and B. S. Park. 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. J. Environ. Biol. 30: 609–614.

Kaufman, P. E., and C. J. Geden. 2009. Development of *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) on live and freeze-killed house fly (Diptera: Muscidae) pupae. Fla. Entomol. 92: 492–496.

Kováčik, P., M. Kozánek, P. Takáč, M. Galliková, and L. Varga. 2010. The effect of pig manure fermented by larvae of house flies on the yield parameters of sunflowers (*Helianthus annus* L.). Acta Univ. Agric. Silvic. Mendel. Brun. 58: 147–153.

Moon, R. D., J. L. Hinton, S. D. O'Rourke, and D. R. Schmidt. 2001. Nutritional value of fresh and composted poultry manure for house fly (Diptera: Muscidae) larvae. J. Econ. Entomol. 94: 1308–1317.

Ogunji, J., R.-U.-A. Summan Toor, C. Schulz, and W. Kloas. 2008. Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turk. J. Fish. Aquat. Sci. 8: 141–147.

Pastor, B., H. Čičková, M. Kozánek, A. Martínez-Sánchez, P. Takáč, and S. Rojo. 2011. Effect of pupa size, oviposition substrate, and adult density on egg production of *Musca domestica* (Diptera: Muscidae). Eur. J. Entomol. 108: 587–596.

Sherrod, P. H. 2010. NLREG user's guide. Phillip H. Sherrod, Brentwood, TN.

Sorokoletov, O. N. 2006. Technological and ecological aspects of waste processing of poultry and swine farming by the larvae of *Musca domestica.* Ph.D. thesis. Novosibirsk State Agrarian University, Russia.

Strange, P. H. 1961. The spectral sensitivity of *Calliphora* maggots. J. Exp. Biol. 38: 237–248.

Tobin, P. C., and C. W. Pitts. 1999. Flotation method for extracting insects from poultry manure samples. J. Med. Entomol. 36: 121–123.

Yang, H., D. Xu, J. Wu, and C. Xue. 2004. Effect of ecological treatment by *Musca domestica* larvae to pig manure on the oviposition and larvae hatching rate. Chin. J. Parasitol. Parasit. Dis. 22: 9–10.

Wingrove, J. A., and P. H. O'Farrell. 1999. Nitric oxide contributes to behavioral, cellular, and developmental responses to low oxygen in *Drosophila.* Cell 98: 105–114.

Yaqub, H. 1997. Earthworm and maggot meals as potential fishmeal replacement. (http://hdl.handle.net/1834/1268).

Zar, J. H. 2010. Biostatistical analysis, 5th ed. Pearson, NJ.

Zuidhof, M. J., C. J. Molnar, F. M. Morley, T. L. Wray, F. E. Robinson, B. A. Khan, L. Al-Ani, and L. A. Goonewardene. 2003. Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. Anim. Feed Sci. Tech. 105: 225–230.

Received 22 June 2011; accepted 7 October 2011.