Bulgarian Journal of Agricultural Science, 24 (No 2) 2018, 279–284
Agricultural Academy

STUDYING DEVELOPMENT OF *HERMETIA ILLUCENS* FLY LARVAE CULTIVATED ON HIGH CELLULOSE PLANT SUBSTRATES

OLGA V. YURINA*; VASILIJ P. KARAGODIN
Plekhanov Russian University of Economics, 117997, Moscow, Russian Federation

Abstract

Yurina, O.V. and V.P. Karagodin, 2018. Studying development of *Hermetia illucens* fly larvae cultivated on high cellulose plant substrates. *Bulg. J. Agric. Sci.*, 24 (2): 279–284

Hermetia illucens L. larvae with experimentally confirmed morphological and genetic characteristics were grown on plant substrates with 4.3% to 19.0% cellulose content: crushed corn kernels, wheat bran, beet pulp, and distillery stillage. It has been determined that the larvae are able to grow rapidly on the plant substrates during 10-14 days until the prepupal stage if optimal conditions are maintained, i.e.: air temperature (28°C) and substrate humidity about 60%. The highest substrate conversion was demonstrated for corn kernels and was equal to 77% in 14 days. A biomass yield of 181 g from 1 kg of substrate was obtained. With wheat bran the conversion was 64%, process time: 10 days. Distillery stillage and beet pulp contained high amounts of cellulose, and their use as feed substrate yielded little accumulation of larvae weight: 84 g and 34 g of dry biomass from 1 kg of substrate, respectively. Presumably, cellulose is a limiting factor as a nutritional medium for *Hermetia illucens* larvae, but bioconversion efficiency in the case of its high content may be increased by adding more nutritious substrates like corn kernels.

Key words: fly; *Hermetia illucens* L.; larvae; Black soldier fly; biomass; bioconversion; substrates; cellulose

Introduction

Presently in some countries of America, Europe, Asian region, Africa, New Zealand, and Australia there is growing attention to insects because of a possibility of growing their mass in vitro and using in feeding stuffs of livestock and fish (Diclaro and Kaufman, 2009). A leader of industrial breeding is *Hermetia illucens* (Diener et al., 2011), which is explained by peculiar biological features of the insect. Black soldier fly *Hermetia illucens L.* is a fly of the family *Stratiomyidae* (*Diptera*); the species is widely distributed in tropical and warm temperature areas (Diener et al., 2009). Adult flies are not attracted by human dwellings, they are not considered vectors; the larvae are easy to keep and able to develop in a wide range of temperatures (20°C to 45°C) and humidity (45% to 90%). High feeding capacity of the larvae allows them to consume various organic substrates, and the yielded larvae biomass may contain up to 40% of protein and fat, which determines their high forage value. Usage of a wide spectrum of organic substrates including agricultural waste as the feeding substrate for cultivation of larvae of this fly determines the practical interest in this species, also in respect of biological management of solid organic waste.

Despite numerous publications about the black soldier fly, there are many specific features in theory as well as practice of cultivating larvae biomass on different breeding media that still remain understudied. As plant wastes typically contain cellulose, there is interest in the issues associated with evaluation of biological conversion by *Hermetia illucens* larvae feeding substrates with different contents of this ingredient.

The aim of this work is to study the peculiarities of development of *Hermetia illucens* larvae cultivated on several plant substrates, including the genetic identification of popu-

**Corresponding author:* olga32661@mail.ru

lation, general characteristics of the larvae properties, and utilization performance of feeding substrates.

Materials and Methods

Species identification of *Hermetia illucens* contained in typical laboratory conditions was made by morphological characteristics, specifics of physiology and behavior (Hajibabaei et al., 2007), as well as by genetic characteristics. Morphological examinations of the larvae were conducted with the use of CamScan MV2300 (Czech Republic) scanning electronic microscope. Molecular-genetic identification of *Hermetia illucens* was made by analyzing CO1 mitochondrial gene fragment (Hebert et al., 2003; Lee et al., 2011). Polymerase chain reaction (PCR) was conducted with the use of a kit of MasterMix x 5 and HotTaq_polimerase (Dialat, Russia) with LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA-3') universal primers. Nucleotide sequence on the examined mtDNA fragment was determined by AB 3500 genetic analyzer (Applied Biosystems, USA); BigDye Terminator v.3.1 kit (Applied Biosystems, USA) was applied with the forward and reverse primers used during the PCR. Species identification was performed using BLAST software included in the international genetic data base GenBank, ncbi. The feeding substrates selected for the examination were wheat bran, dried wheat distillery stillage, beet pulp, and crushed corn kernels normally used in animal foods (Table 1).

To ensure standardized larvae breeding conditions, the initial substrates of identical humidity (about 60%) were poured in a 5-7 cm layer into plastic containers. The substrates were populated with larvae 6 days old. The larvae planting density was identical for all substrates and equaled to 5 specimens per 1 cm^2. The air temperature inside the room was maintained at 28±1°C. To analyze the larvae weight, they were screened off, weighted on electronic scales, dried at 70°C, and then the dry weight was determined. Residual mass of the initial substrate upon completion of the biological conversion was determined after drying out at 105°C. The end of the substrate biological conversion process was identified by appearance of dark-brown prepupae among the lighter-colored larvae. To determine the prepupal content, a sample of substrate was taken (125 cm^3), and the share of prepupae in the total quantity of larvae was calculated. The substrate larval treatment stage was considered complete if the share of prepupae was 50% or higher. The following values were determined by calculation: total substrate consumption as the ratio of the difference between the initial dry substrate mass and the final dry residue (substance consumption) to the initial dry mass, in grams and in %; daily substrate consumption rate in g/days as the ratio of the substance consumption to the larval stage duration; daily substrate consumption per unit of surface area in g/day/cm^2 as the ratio of the substance consumption per day to the area occupied by the substrate; larvae mass gain as the difference between the final and the initial dry mass of the larvae in grams; daily mass gain of the larvae in g/day as the ratio of the larvae mass gain to the larval stage duration. Validity of the results was estimated using the Student's t-test.

Results

The insects used in the work matched all morphological properties of *Hermetia illucens*. As a result of molecular genetic analysis (Table 2), sequences of CO1 mtDNA mitochondrial gene fragment 657 bps long were obtained, which, when compared with homologous sequences from the GenBank, demonstrated high (99%) similarity of the tested specimen to *Hermetia illucens* (Table 2). The nucleotide sequence of *Hermetia illucens* is registered in the GenBank, specimen h-il1 no. KY817115.

Basic indicators of the larvae development on feeding substrates are shown in Table 3.

A benchmark experiment was carried out to compare the larvae development indicators on corn kernels and wheat bran as the most promising substrates for effective accumulation of larvae biomass within a short period of time. The findings of the experiment are shown in Table 4.

Morphological examination of the larva integument in the case of developing inside the feeding substrate has demonstrated a complex surface structure (Figure 1). The body of a larva consists of segments covered with spines and adhered coarse foreign lumps. The surface of the segments is

Table 1
Approximate composition of vegetable substrates used for the larvae cultivation

Substrate	Protein (%)	Fat (%)	Cellulose (%)	Ash (%)
Corn grits	9.2	4.3	4.3	1.3
Wheat bran	15.1	4.1	8.8	5.8
Distillery stillage	20.1	7.6	10.5	8.6
Beet pulp	7.7	0.5	19.0	6.0

Table 2
Degrees of similarity of the nucleotide sequence obtained in the cytochrome oxidase gene fragment of 1 mitochondrial DNA subunit of the examined specimen of fly with homologous sequences from the GenBank

GenBank sequence description (species, isolate name, genome fragment)	Degree of similarity to the reference sequence	GenBank sequence no.
Hermetia illucens isolate Yangyang 2 cytochrome oxidase subunit I gene, par- tial cds; mitochondrial	99%	FJ794367.1
Hermetia illucens isolate Bonghwa 1 cytochrome oxidase subunit I gene, partial cds; mitochondrial	99%	FJ794375.1
Hermetia illucens voucher Gurae-10 cytochrome oxidase subunit I gene, partial cds; mitochondrial	99%	HQ541184.1
Diptera sp. KMGHap_101 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	97%	JQ344949.1
Palaeosepsis pusio voucher su33 cytochrome oxidase subunit I (COI) gene, par- tial cds; mitochondrial	85%	EU435797.1
Stegana mengla voucher SCAU 120652 cytochrome oxidase subunit I gene, partial cds; mitochondrial	85%	HQ260646.1

Table 3
Results of *Hermetia illucens* larvae development on feeding substrates

Substrate	Duration of substrate processing by the larvae (days)	Conversion (%)	Larvae biomass yield from 1 kg of substrate (kg)	Larvae biomass yield from 1 m^2 (kg)
Corn grits	14	77.4±1.6	0.181±0.009	3.15±0.08
Corn grits + 10% of wheat bran	14	85.7±2.2	0.242±0.011	3.30±0.09
Wheat bran	10	63.6±1.5	0.145±0.006	1.45±0.03
Distillery stillage	14	52.7±1.3	0.084±0.004	0.74±0.05
Beet pulp	30	75.6±1.6	0.034±0.007	0.30±0.02

Table 4
Results* of *Hermetia illucens* larvae development on crushed corn kernels and wheat bran at the planting density of 5 specimens per 1 cm^2 and the layer height of 5-7 cm

Characteristics	Feeding substrate	
	Wheat bran	Crushed corn kernels
Initial substrate mass (g)	191.0±0.3	315.0±0.2
Final substrate mass (g)	69.5±2.2	71.2±2.8
Consumed substrate (g)	121.5±6.7	243.8±7.7
Total substrate consumption (%)	63.6	77.4
Daily substrate consumption (g/day)	12.1±0.7	17.4±0.5
Daily substrate consumption per unit of surface area (g/day/$cm^{2)}$	0.06±0.002	0.09±0.003
Initial total larvae mass (g)	6.2±0.8	6.3±0.7
Final total larvae mass (g)	43.0±0.6	56.7±0.7
Total larvae mass gain (g)	36.8±3.6	50.4±3.2
Daily larvae mass gain (g)	3.7±0.9	3.6±1.1
Final mass of 1 larva (mg)	43.4 ±0.6	54.3±1.9
Humidity (%)	57.8±5.7	58.3±6.5
Duration of larval stage (days)	16	20

*All weights are represented in terms of dry mass

covered with sclerotized integument buildups, with microscopic foreign particles accumulated in-between.

Discussion

The findings have shown that the larvae are able to develop rapidly in artificial environments on vegetable substrates with the air temperature maintained at 28°C and the substrate humidity kept at 60%. Metagenomic methods of analyses have shown presence of a cellulose gene in the intestinal microbiota of black soldier fly larvae (Nguyen et al., 2013), which determines their principal ability to grow on a vegetable substrate. Besides, several enzymes have been identified in *H. illucens* that assist in breaking down the three

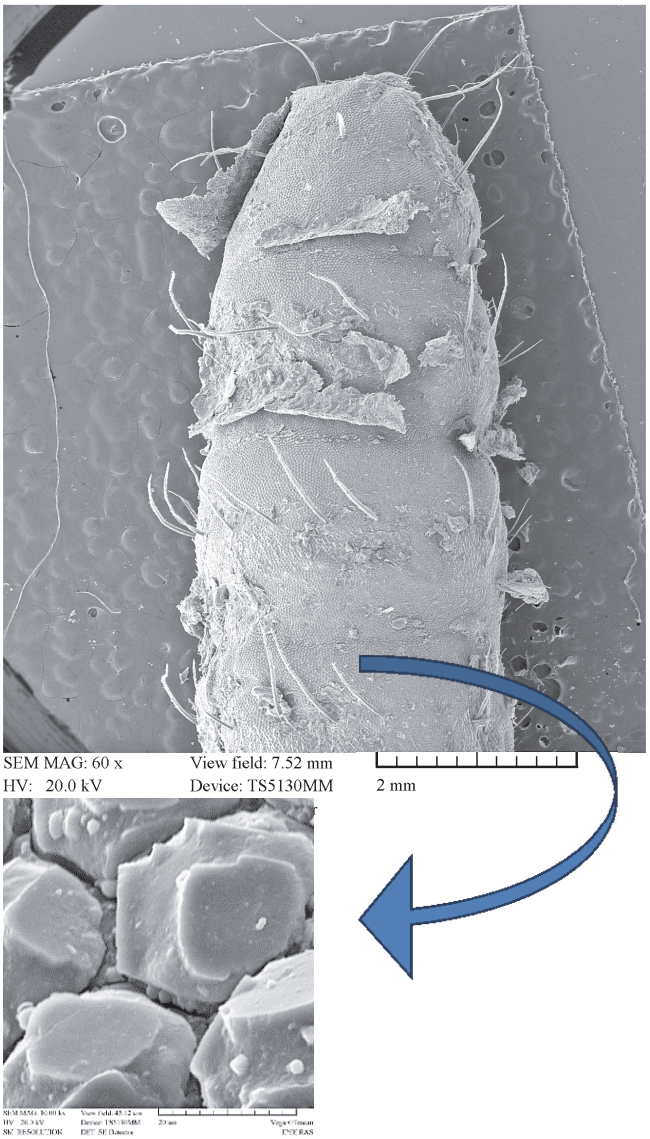

Fig. 1. Microphoto of body fragment of *Hermetia illucens* larvae: A - body fragment, SEM MAG: 60x; B - sclerotized structures of the external cover, SEM MAG: 10.00kx

major macronutrients using amylases, proteases and lipases (Kim et al., 2011; Park et al., 2013).

However, our work has revealed that the content of poorly digestible cellulose is a limiting factor for obtaining maximum biomass of the larvae. Distillery stillage and beet pulp, which both contain substantial amounts of cellulose (10% to 19%), when used as a feeding source, tend to yield insignificant amounts of biomass: 0.084 and 0.034 kg of dry biomass from 1 kg of substrate, respectively, despite the relatively high mass fraction of protein and fat in the distillery stillage and of sugars in the beet pulp. Although the conversion of beet pulp reached 75.6%, the process was very long – 30 days. For comparison, corn kernels yielded 0.181 kg of dry biomass from 1 kg of crushed kernels in 14 days with 77.4% substrate conversion.

Wheat bran over the larval stage period were consumed by 63.6% in 10 days, which turned out lower than the similar value in the case of corn grits, and yielded lesser accumulation of the larvae biomass. However, in combination with corn kernels, the mixture of bran with corn kernels was better balanced in terms of nutritious elements and yielded a larvae biomass 33.7% higher than with the use of corn kernels for the same period of time. It is pertinent to note that bioconversion seems to be an effective and simple method for calculating feeding efficiencies of the larvae for industrial reasons (Leong et al., 2015; Li et al., 2011; Paz et al., 2015; Surendra et al., 2016).

An important index of the insects breeding process is the daily substrate consumption and the biomass gain that characterizes the speed and the amount of accumulation of the final mass by the larvae. In the benchmark experiment of breeding larvae on corn kernels and wheat bran it was shown that the daily consumption of wheat bran was lower than that of corn kernels (12.1 and 17.4 g/day respectively), which impacted the final conversion: the feeding substrate residue was greater for the bran than for the corn kernels. And although the daily larvae mass gain on the bran was rather similar to the larvae mass gain on the corn kernels (3.7 and 3.6 g/day), yet the final mass of a single larva on the bran was lower (43.4 mg vs. 54.3 mg on the kernels), as was the total larvae mass gain: 36.8 g on the bran and 50.4 g on the corn kernels with the same planting density of 5 specimens per cm^2. Larval mass showed a strong trend with bioconversion and waste reduction but has its own merits as it gives a more individual look at how larvae performed compared to the population approach that feeding efficiencies use. It is seen that larval mass trends negatively as feed depth increases and this may be due to overcrowding stress not allowing the larvae to metabolise as the access to feed and the space they could feed in changes dramatically (Applebaum and Heifetz, 1999).

With identical breeding conditions, the larval stage of *Hermetia illucens* was finished with transition into the prepupal stage in the case of growing on wheat bran faster (in 10 days), whereas on corn grits this process was longer (14 days). The endocrine system and the activity of neurosecretory cells of the larvae are greatly affected by external

factors, especially by the conditions of nourishment. In our experiment, the cultivation of *Hermetia illucens* larvae on the more nutritious substrate – corn grits – resulted in longer duration of the larval stage, whereas the development on high-cellulose wheat bran with lesser amount of digestible protein and carbohydrates promoted faster transition from the larval stage to the prepupal stage.

It has been demonstrated that *Hermetia illucens* larvae can be suitable for bioconversion of solid agricultural vegetable wastes in the conditions of north latitude (Moscow) in enclosed rooms with the environmental parameters maintained as appropriate. Similar results for *Hermetia illucens* were known because of their ability to reduce the amount of waste present thus acting as manure management agents in confined animal feeding operations (Lalander et al., 2014; Diener et al., 2014). This has been studied to the management of organic wastes such as palm kernel wastes (Arief et al., 2012) and vegetable wastes (Paz et al., 2015).

Hermetia illucens has been repeatedly shown to be a rich source of protein and lipids with a high amino and fatty acid complex that can be manipulated under the correct conditions to exceed feeds in some aspects of their composition, having some of the best compositions among insects for food and feed, making the species of significant interest (Makkar et al., 2014; Van Huis et al., 2015; European Food Safety Authority, 2015). The larvae, prepupae and pupae of *H. illucens* have been successfully fed to fish (Stamer, 2015; Tschirner and Simon, 2015), poultry and other birds (Maurer et al., 2016), swine (Veldkamp and Bosch, 2015). However, when the larvae are developing within an organic substrate, particles of the substrate are sorbed on the larva's surface. These particles, which may contain bacteria, mushroom spores, and other potentially hazardous ingredients, are hard to separate from the insect's surface because of its complicated folded structure. Therefore it seems reasonable to decontaminate the biomass for forage purposes by thermal treatment or to break up the biomass into constituent ingredients obtaining hydrolysates and extracts.

Conclusion

This work has demonstrated the principal possibility of using the culture of a tropical insect – black soldier fly (*Hermetia illucens*) for growing on plant feeding substrates with a high content of cellulose in artificial environment. The mass fraction of cellulose in a feeding substrate is a factor which, on one hand, is limiting the growth of the larva, but on the other hand, it may stimulate the insect's digestion and raise the bioconversion process efficiency. Accumulation of larvae biomass can be increased by making mixtures of feeding substrates. The best option was a mixture of crushed corn kernels and wheat bran.

Acknowledgements

The authors would like to thank the Plekhanov Russian University of Economics for funding this research.

References

Applebaum, S.W. and Y. Heifetz, 1999. Density-dependent physiological phase in insects. *Annual Review of Entomology*, **44**: 317-341.

Arief, M., A.N. Ratika and M. Lamid, 2012. The effect of palm kernel meal and rice bran media combination which are fermented to the production of black soldier fly maggot (*Hermetia illucens*) as a source of fish feed protein. *Jurnal Ilmiah Perikanan dan Kelautan,* **4**: 33-37.

Diclaro, J.W. and P.E. Kaufman, 2011. Black soldier fly *Hermetia illucens Linnaeus* (Insecta: Diptera: *Stratiomyidae*). *University of Florida IFAS Extension*, 1-4.

Diener, S., S. Semiyaga, C.B. Niwagaba, A.M. Muspratt, J.B. Gning, M. Mbéguéré, J.E. Ennin, C. Zurbrugg and L. Strande, 2014. A value proposition: Resource recovery from faecal sludge – Can it be the driver for improved sanitation? *Resources, Conservation and Recycling*, **88**: 32-38.

Diener, S., N. Solano, F. Gutiérrez, C. Zurbrügg and K. Tockner, 2011. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valorization,* **2** (4): 357-363.

Diener, S., C. Zurbrügg and K. Tockner, 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research*, **27**: 603-610.

European Food Safety Authority, 2015. Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, **13**: 1-60.

Hajibabaei, M., G.A. Singer, E.L. Clare and P.D.N. Hebert, 2007. Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. *BMC Biol*., **5**: 24.

Hebert, P.D.N., A. Cywinska, S.L. Ball and J.R. de Waard, 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B, Biological Sciences,* **270:** 313-321.

Kim, W., S. Bae, K. Park, S. Lee, Y. Choi, S. Han and Y. Koh, 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology,* **14**: 11-14.

Lalander, B.V., C.H. Fidjeland, J. Diener, S. Eriksson and B. Vinnerås, 2014. High waste-to-biomass conversion and efficient *Salmonella spp*. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development,* **35:** 261-271.

Lee, D.W., J.B. Seo, M.H. Nam, J.S. Kang, S.Y. Kim, A.Y. Kim, W.T. Kim, J.K. Choi, Y. Um, Y. Lee, I.S. Moon, H.R. Han, S.H. Koh, Y.H. Je, K.J. Lim, S.H. Lee and Y.H. Koh, 2011. A combination of biochemical and proteomic analyses reveals Bx- LEC-1 as an antigenic target for the monoclonal antibody

3-2A7-2H5-D9-F10 specific to the pine wood nematode. *Mol. Cell Proteomics,* **5:** 1-13.

Leong, S.Y., S.R. M. Kutty, C.K. Tan and L.H. Tey, 2015. Comparative study on the effect of organic waste on lauric acid produced by *Hermetia illucens* larvae via bioconversion. *Journal of Engineering Science and Technology,* **8**: 52-63.

Li, Q., L. Zheng, N. Qiu, H. Cai, J.K. Tomberlin and Z. Yu, 2011. Bioconversion of dairy manure by black soldier fly (Diptera: *Stratiomyidae*) for biodiesel and sugar production. *Waste Management,* **31:** 1316-1320.

Makkar, H.P.S., G. Tran, V. Heuzé and P. Ankers, 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology,* **197:** 1-33.

Maurer, V., M. Holinger, Z. Amsler, B. Früh, J. Wohlfahrt, A. Stamer and F. Leiber, 2016. Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. *Journal of Insects as Food and Feed*, **2:** 83-90.

Nguyen, T.T., J.K. Tomberlin and S. Vanlaerhoven, 2013. Influence of resources on *Hermetia illucens* (Diptera: *Stratiomyidae*) larval development. *Journal of Medical Entomology*, **50** (4): 898-906.

Park, K.H., Y.C. Choi, S.H. Nam, W.T. Kim, A.Y. Kim and S.Y. Kim, 2013. Recombinant expression and enzyme activity of Chymotrypsin-like protease from black soldier fly, *Hermetia illucens* (Diptera: *Stratiomyidae*). *International Journal of Industrial entomology*, **25:** 181-185.

Paz, A.S.P., N.S. Carrejo and C.H.G. Rodriguez, 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.), (Diptera: *Stratiomyidae*). *Waste Biomass Valorisation,* **6:** 1059-1065.

Stamer, A., 2015. Insect proteins – a new source for animal feed. *EMBO Reports,* **16:** 676-680.

Surendra, K.C., R. Olivier, J.K. Tomberlin, R. Jha and S. Kumar, 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy,* **1:** 1-6.

Tschirner, M. and A. Simon, 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, **1:** 1-12.

Van Huis, A., M. Dicke and J.J.A. Van Loon, 2015. Insects to feed the world. *Journal of Insects as Food and Feed,* **1:** 3-5.

Veldkamp, T. and G. Bosch, 2015. Insects: A protein-rich feed ingredient in pig and poultry diets. *Animal Frontiers*, **5:** 45-50.

Received August, 3, 2017; accepted for printing March, 23, 2018