An Artificial Diet for Rearing *Cochliomyia macellaria* (Diptera: Calliphoridae)

M. F. CHAUDHURY[1,2] AND S. R. SKODA[3]

J. Econ. Entomol. 106(4): 1927–1931 (2013); DOI: http://dx.doi.org/10.1603/EC12361

ABSTRACT Larvae of the secondary screwworm, *Cochliomyia macellaria* (F.), feed on carrion and may sometimes cause animal myiasis. They have been reared in the laboratory on various animal tissues to study their growth and development because of their importance in forensic science. We use secondary screwworms in our laboratory for preliminary experiments as a model for the primary screwworm, *Cochliomyia hominivorax* (Coquerel), which has been eradicated from the United States. *C. macellaria* larvae reared on animal tissues produced a putrid odor, an unfavorable condition in the laboratory, and variable pupal size and weight, bringing into question the validity of use as a model for *C. hominivorax*. Therefore, studies were conducted to develop a less odiferous artificial diet with reduced variability in resulting pupae by comparing three diets: 1) an artificial diet prepared from spray-dried blood, spray-dried poultry egg, dry milk substitute, and solidified with a polyacrylate polymer gel; 2) a fresh blood-based diet prepared similarly, except fresh bovine blood was used in place of spray-dried blood; and 3) a beef liver diet. Data from seven life-history parameters of resulting insects were collected and analyzed. Larval and pupal weights of *C. macellaria* reared on both the dry and fresh blood-based diets were significantly higher than those reared on the liver diet. Numbers of pupae and percentage of adult emergence were also significantly higher from both dry blood and fresh blood-based diets than those reared on the liver diet. Female flies developing from larvae reared on dry and fresh blood-based diets laid significantly more eggs than females developing from larvae reared on the liver diet. Results show that *C. macellaria* larvae developed and grew normally in the dry and fresh blood-based diets, indicating that an artificial diet can effectively replace the liver diet commonly used for rearing *C. macellaria*.

KEY WORDS carrion, forensic entomology, myiasis, secondary screwworm, spray-dried blood

The secondary screwworm, *Cochliomyia macellaria* (F.), feeds primarily on carrion and may act as a secondary agent for myiasis in animals. Adults are attracted to various decaying organic matters for food and reproduction, including animal and human feces, carcasses, and wounds (Hall 1948, Zumpt 1965). Because of its necrophagous (feeding on corpse) behavior, *C. macellaria* is considered to be an important species in forensic entomology on which to base postmortem interval estimations (Amendt et al. 2004). This blow fly is valuable as a forensic tool because its succession and occurrence on decomposing cadavers have been well defined. *C. macellaria* is considered a facultative parasite, sometimes present as a secondary invader in myiasis cases, generally feeding on the edge or surface of a wound (Harrison and Pearson 1968).

The secondary screwworm has been reared in the laboratory by using various animal tissues, including horse meat, beef liver, porcine tissue, and various other tissue components (Byrd and Butler 1996, Aguilar–Cuelho and Milward–de-Azevedo 1998, Boatright and Tomberlin 2010). Byrd and Butler (1996) reported that 80% of the adults and larvae preferred lean porcine meat. In some of the experiments in our laboratory, we have been using *C. macellaria* as a model insect in place of the primary screwworm, *Cochliomyia hominivorax* (Coquerel), which has been eradicated from the United States and cannot be reared in the laboratory in the United States without proper bio-secure facilities. *C. hominivorax* larvae are currently reared on an artificial diet in our laboratory in Panama. In our laboratory in Lincoln, NE, we have been rearing *C. macellaria* larvae by using beef or chicken liver. However, this method of rearing has not been suitable for several reasons, including production of variable pupal size and weight as well as the foul odor in the insectary emitted from the decomposing liver. Consequently, we conducted a series of experiments toward developing an artificial larval rearing medium that can support larval growth and development, resulting in homogeneous and high-quality in-

Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. USDA is an equal opportunity employer and provider.

[1] USDA–ARS, Screwworm Research Unit, 109 C Entomology Hall, University of Nebraska–Lincoln, Lincoln, NE 68583.

[2] Corresponding author, e-mail: muhammad.chaudhury@ars.usda.gov.

[3] USDA–ARS, Screwworm Research Unit, KBUSLIRL, 2700 Fredericksburg Rd., Kerrville, TX 78028.

sects without objectionable odor from the rearing system.

Unlike *C. macellaria*, *C. hominivorax* feeds on living animal tissue, and successful artificial diets have been developed for primary screwworm by using spray-dried blood, spray-dried poultry eggs, and powdered milk (Taylor et al. 1991, Chaudhury et al. 1999, Chaudhury and Skoda 2007). A gelling agent is used to solidify the diet to provide desired viscosity and texture. Harris et al. (1984, 1985) tested several gelling compounds to solidify the larval rearing medium. Among these compounds, the gelling agent Water-Lock G-400 (Grain Processing Corporation, Muscatine, IA) was found most suitable and was later used in the larval diet for mass rearing (Taylor and Mangan 1987, Taylor et al. 1991). The consistency of the diet mixed with Water-Lock remains relatively constant over a wide range of humidities, the diet remains semisolid, emits little odor, and is acceptable to screwworm larvae for a longer time before becoming contaminated by toxic waste products (Taylor 1988).

We report here the development of a gel-based artificial diet for *C. macellaria*, prepared from spray-dried bovine blood, spray-dried poultry eggs, and a dry milk substitute, and compared it with the commonly used beef liver diet with respect to several life-history parameters, including larval and pupal weight, adult emergence and longevity, fecundity, and fertility over six generations. Comparisons were also made between the artificial diet and another diet where spray-dried blood was replaced with fresh bovine blood.

Materials and Methods

Insects and Dietary Ingredients. The *C. macellaria* colony maintained in the U.S. Department of Agriculture–Agriculture Research Service (USDA–ARS) laboratory, Lincoln, NE, originated from pupae obtained from a laboratory colony developed in the USDA–ARS laboratory, Fargo, ND. Dietary ingredients used for preparing the artificial diet were obtained from Nutrient Concepts, Stockton, CA (spray-dried whole bovine blood and spray-dried poultry egg powder), and Calva Products, Acampo, CA (dry milk substitute). Sodium polyacrylate gel, Water-Lock G-400, was obtained from Grain Processing Corporation, Muscatine, IA. Fresh bovine blood was collected from a local slaughter house, which was mixed with anticoagulant sodium citrate (0.4 g per 100 ml blood). Beef liver was purchased from a local retail store (Hy-Vee Stores Inc., Lincoln, NE).

Diet Preparation. The dry blood-based diet was prepared by weighing and mixing all dry ingredients as follows: 50 g spray-dried blood, 50 g spray-dried egg powder, 40 g dry milk substitute, 12 g sodium polyacrylate gel. One milliliter of formalin (37% formaldehyde solution) was then added to the dry mixture. Warm tap water (≈850 ml at 40°C) was then added to the mixture to make 1 liter of diet (Chaudhury and Alvarez 1999). The fresh blood-based diet was prepared by mixing 150 ml of fresh blood, 60 g of spray-dried egg powder, 40 g of milk substitute, and 25 g of gel with water to make 1 liter of diet (Chaudhury et al. 2011). Each diet was hand-mixed in a plastic container for 5 min to obtain a viscosity of ≈7,000 cP (measured with a rotational digital viscometer, Cole Parmer, Vernon Hills, IL). Beef liver diet was prepared by cutting 1-cm-thick slices of liver into 5 by 10-cm pieces, keeping the slices immersed in blood that came with the liver and leaving the container at room temperature for at least an hour before using for the experiment. Preliminary tests were conducted to determine appropriate container size, number of eggs per container, and the amount of diet and viscosity for optimum larval development and survival.

Experiments. Experiments were conducted according to the protocols described by Chaudhury and Skoda (2007), with some modifications in procedures and containers used. Tests were set up by placing 0.5 liter of one of the two freshly prepared diets or 450 g of sliced liver with accompanying blood in a plastic rearing tray (20 by 18 by 10 cm). Approximately 50 mg of secondary screwworm eggs (≈1,000 eggs) was placed directly on the top of the test diet. The rearing pan was then placed inside a larger plastic pan (35 by 30 by 12 cm) containing ≈1-cm-deep vermiculite. The large plastic pan with the rearing tray containing the diet and eggs was then placed on a rack in the rearing room (28 ± 2°C and 60 ± 5% relative humidity). Three trays of each diet using eggs from three different adult cages were set up. After the hatching of eggs (next day; ≈24 h after egg placement), the egg shell masses were removed from the top of the diet, washed with 1% sodium hydroxide solution to separate the empty shells, and microscopically examined to ensure normal hatch (at least 80% hatch in each case). On the following day, another 0.5 liter of freshly prepared diet (or 450 g liver) was added to each of the trays with developing larvae. Approximately 4.5 d after hatching, the mature larvae crawling off of the diet were collected in vermiculite below the rearing tray. A sample of 100 mature larvae was collected randomly and weighed to determine mean individual larval weight. These larvae were then returned to their respective trays. All larvae that crawled-off were allowed to pupate in the vermiculite. Once pupated, 100 pupae were collected randomly and weighed to determine mean individual pupal weight. All pupae were sifted from the vermiculite, and the total weight of all the pupae was determined. The number of pupae per tray was determined by dividing the weight of total pupae by the weight of 100 pupae and multiplying the resulting weight with 100.

One hundred pupae from each tray were collected and held in small emergence cages prepared from clear plastic cups (9 cm base diameter by 11 cm lid diameter by 7 cm height) with perforated lids. Percent adult emergence was recorded. Adult longevity was determined by holding 100 newly emerged flies in small screened cages (10 by 10 by 10 cm) with 10 g sugar provided in a petri dish and water in a bottle with a dental wick. Mortality was recorded until 50% of the flies survived. To determine fecundity, 100 females were collected 10 d after emergence from cages of flies

Table 1. Comparison of biological parameters of *C. macellaria* reared on artificial diets based on dry blood, fresh blood, and a standard liver diet (mean ± SE)

Parameters	*F* values, *P* (df = 2, 51)	Dry blood diet	Fresh blood diet	Liver diet
Larval wt (mg)	7.01*, 0.002	65.63 ± 0.93a	66.92 ± 1.01a	62.31 ± 0.73b
Pupal wt (mg)	27.96*, 0.000	45.31 ± 0.75a	46.37 ± 0.33a	40.89 ± 0.49b
Pupae per tray	59.13*, 0.000	794.7 ± 10.6a	828.3 ± 10.1a	642.6 ± 16.8b
% emergence	8.42*, 0.001	95.27 ± 0.83a	97.67 ± 0.6a	92.66 ± 1.08b
Eggs laid (mg)	3.6*, 0.035	693.1 ± 33.7a	720.8 ± 23.1a	626.4 ± 17b
% egg hatch	1.5, 0.244	90.89 ± 0.52	90.75 ± 0.72	89.67 ± 0.49
Adult longevity (d)	3.06, 0.056	17.88 ± 0.4	18.06 ± 0.63	16.44 ± 0.44

Means in the same parameter of the diets (row) not followed by the same letter are significantly different from each other by Tukey's comparison test after significant *F* values (*) ($P = 0.05$).

emerged from pupae collected from each test diet. Female flies were introduced in small screened cages (10 by 10 by 10 cm) and allowed to oviposit in a petri dish with a piece of liver (≈20 g) placed in the cage. After allowing ≈3 h for oviposition, eggs were collected and weighed. To determine fertility, a sample of 50 mg of eggs from each experiment was placed on 5 g of liver in a petri dish lined with moist filter paper and allowed to hatch. The unhatched eggs and the eggshells from each dish were collected, counted, and percent hatch determined. Each test was repeated for six consecutive generations.

Statistical Analysis. Data were statistically evaluated with analysis of variance by using complete randomized block design followed by Tukey–Kramer multiple comparison tests to separate means after a significant *F* value (Sokal and Rohlf 1981, Number Cruncher Statistical System [NCSS] 2000).

Results

Data collected from seven life parameters—larval weight, pupal weight, number of pupae, percentage of adult emergence, fecundity, percentage of egg-hatch, and adult longevity—were compared for the three diets (Table 1). Larvae crawled off from the diet trays ≈4.5 d after the egg-hatch, although the larvae from liver diet tray started crawling out 2–3 h earlier than the larvae of the other two diets. Larval and pupal weights of the insects reared on both blood-based diets were significantly higher than those obtained from insects reared on liver diet. These two parameters did not vary significantly between the two blood-based diets. The number of pupae per tray was significantly higher in both blood-based diets than the liver diet. Percentages of adult emergence from the pupae reared on either blood-based diet were also significantly higher than the adult emergence from pupae reared on the liver diet. Female flies resulting from the larvae reared in blood-based diets laid significantly more eggs than the females from larvae reared on the liver diet. There were no significant differences among the percentages of egg-hatch from the eggs of flies reared from all three diets. Similarly, adult longevity in days was not significantly different from each other (Table 1).

Results of the life-history parameters of screwworms reared in all three diets for six consecutive generations are presented in Table 2. Larval weight, pupal weight, number of pupae per tray, and percentages of adult emergence were similar in all six generations. The number of eggs deposited by the fifth-generation females from larvae reared in dry ingredients-based artificial diet was significantly higher than the eggs deposited by the females of other generations. Similarly, the number of eggs deposited by the fifth- and sixth-generation females obtained from larvae reared in fresh blood-based diet was significantly higher than the number of eggs deposited by the females of other generations. The lowest amount of eggs was collected from the third-generation females obtained from the larvae reared in both these diets. The number of eggs laid by the females obtained from larvae reared in beef liver did not vary significantly through the generations. Percentage of egg-hatch did not vary, except some significantly low hatch in the egg batches obtained from first- and fourth-generation females originating from the larvae reared in fresh blood-based diet. In all three diets, the adult longevity was significantly longer in the fifth and sixth generations compared with other generations.

Discussion

Results clearly show that larval growth and development is supported by the diets tested, indicating that an artificial diet can effectively replace the liver diet that is commonly used for rearing *C. macellaria.* This is the first report of a successful artificial diet for this species. Several formulations of artificial diets are available for the primary screwworm, *C. hominivorax* (Harris et al. 1984; Taylor 1988; Taylor and Mangan 1987; Chaudhury et al. 1998, 2002; Chaudhury and Alvarez 1999; Chaudhury and Skoda 2007). However, these diets were previously found unsuitable for *C. macellaria* larvae. These larvae reared on *C. hominivorax* gel-based diet suffered high mortality, and those that survived resulted in low-weight larvae and pupae (M.F.C., unpublished data). This was probably because of the specific viscosity of the diet required for the primary screwworm. Chaudhury and Skoda (2009) reported a viscosity range for primary screwworm diet to be between 4,000 and 5,000 cP (comparable with thick ketchup). Both dry and fresh blood diets used in this investigation for *C. macellaria* maintained a viscosity of 7,000 cP at 27°C (comparable with

Table 2. Biological parameters of *C. macellaria* reared on dry blood-based diet (A), fresh blood-based diet (B), and beef liver (C) through six generations (mean ± SE)

Parameters	Diet	*F* values, *P* (df = 5, 12)	Generations					
			1	2	3	4	5	6
Larval wt (mg)	A	0.3, 0.902	65.7 ± 0.2	64.6 ± 3	66.6 ± 2.1	67.3 ± 3.3	63.4 ± 2.6	66.2 ± 2.7
	B	1.88, 0.172	65.5 ± 0.8	65.9 ± 3.6	67.3 ± 0.7	63.1 ± 1.8	67.5 ± 3.5	72.2 ± 0.3
	C	0.51, 0.763	60.7 ± 1.1	62.3 ± 2.4	61.8 ± 2.9	63.7 ± 0.6	61.4 ± 1.5	64.6 ± 2
Pupal wt. (mg)	A	0.47, 0.792	44.3 ± 1	44.5 ± 3	47.1 ± 0.9	44.3 ± 1	44.5 ± 3.2	47.1 ± 0.9
	B	0.22, 0.946	45.9 ± 0.3	46.4 ± 1.4	46.8 ± 0.8	45.9 ± 0.3	46.4 ± 1.4	46.8 ± 0.8
	C	2.92, 0.059	39.2 ± 0.6	40.9 ± 1.1	39.3 ± 1.8	41.6 ± 0.8	40.7 ± 0.3	43.6 ± 0.6
Pupae per tray	A	1.7, 0.209	799 ± 34	743 ± 23	810 ± 7	805 ± 10	778 ± 38	833 ± 9
	B	1.5, 0.261	833 ± 24	816 ± 13	803 ± 46	866 ± 2	807 ± 6	857 ± 16
	C	0.5, 0.763	634 ± 47	647 ± 34	602 ± 43	629 ± 73	699 ± 28	646 ± 23
% emergence	A	0.7, 0.653	97 ± 1.8	95 ± 2.9	93 ± 1.5	97 ± 1.7	95 ± 2.9	94 ± 1.5
	B	2.8, 0.063	95 ± 0.9	99 ± 0.7	99 ± 0.7	96 ± 2.2	99 ± 0.7	95 ± 0.7
	C	0.8, 0.577	95 ± 1.5	90 ± 1.9	92 ± 4	95 ± 2.5	90 ± 2.7	93 ± 3.8
Eggs laid (mg)	A	4.4*, 0.017	722 ± 19b	599 ± 124b	536 ± 33c	653 ± 35b	872 ± 17a	776 ± 48b
	B	3.2*, 0.045	740 ± 73ab	666 ± 34b	622 ± 47c	678 ± 36b	823 ± 33a	796 ± 20a
	C	0.9, 0.498	670 ± 13	572 ± 69	596 ± 43	661 ± 14	654 ± 28	606 ± 54
% hatch	A	0.3, 0.917	91 ± 1.2	90 ± 1.2	91 ± 1.9	90 ± 1.2	91 ± 1.2	92 ± 1.9
	B	20*, 0.000	87 ± 0.6b	93.7 ± 0.9a	92 ± 0.6a	87 ± 0.6b	93.7 ± 0.9a	92 ± 0.6a
	C	1.3, 0.326	90.3 ± 1.9	88 ± 0.6	90.7 ± 0.3	90 ± 1.9	88 ± 0.6	90.7 ± 0.3
Adult longevity (d)	A	3.8*, 0.027	17 ± 0.6ab	17.3 ± 0.3ab	16.3 ± 0.9b	17.7 ± 0.9ab	20.3 ± 0.9a	18.7 ± 0.7a
	B	9.4*, 0.000	15.7 ± 0.9b	15 ± 0.6b	17.3 ± 0.3ab	18.3 ± 0.9ab	21.3 ± 1.2a	20.7 ± 0.9a
	C	5.4*, 0.008	17 ± 1ab	14.3 ± 0.3b	14.7 ± 0.3b	16.3 ± 0.9ab	18 ± 0.6a	18.3 ± 0.9a

Means in the same parameter of the diets not followed by the same letter were significantly different from each other by Tukey's comparison test after significant *F* values (*) ($P = 0.05$).

a soft gelatin). The formulation that was used in this investigation was most suitable for *C. macellaria* larval growth and development, which produced acceptable size and number of pupae. Pupae obtained from the larvae reared on the dry blood artificial diet were significantly larger in size, probably because of the homogeneous nature of the diet that provided all necessary nutrients supplied by the three dietary ingredients used. The possible cause of lower mean weight for pupae from the liver diet could be because of early crawl-off of some larvae, as noted from the liver diet tray than that from the two gel-based diets. Minor differences in some of the life-history parameters through six generations of rearing indicate that the diet formulations are satisfactory for prolonged rearing of *C. macellaria.* Decrease of egg production during the third generation for both the gel-based diets, followed by significant increase during the next consecutive generations, indicates that this phenomenon is temporary and not a gradual decline in egg production. Significant reduction in egg-hatch for the first- and fourth-generation eggs from the blood-based diet was not expected and could be an experimental error. Reason for increase in adult longevity during the fifth and sixth generations for all diets is not clear but may be because of gradual adaptation to the environment used for experimentation.

The dry blood-based artificial diet was found most suitable for use in the laboratory because of the production of much less odor in comparison with liver or fresh blood-based diet. The gelling agent probably absorbed much of the metabolic wastes and, thus, reduced the odor emitted during larval rearing. However, if the rearing conditions are such that some odor is tolerable, either blood-based diet would be satisfactory. The dry blood-based diet was simple to prepare and handle. All of the dry ingredients used to prepare the diet are commercially available from the indicated vendors, they are less expensive to purchase in bulk, and can be stored in a cool and dry place for an extended period with no apparent loss of nutritional quality. The current study used dry dietary ingredients purchased 8 mo before the start of experiments and the gelling agent was purchased 6 yr ago.

Acknowledgments

We thank Steve Danielson, Frank M. Davis, and Donald B. Thomas for reviewing the manuscript. Technical assistance of Kim Tangtrakulwanich is gratefully acknowledged. This article reports the results of research only.

References Cited

Aguilar–Cuelho, V. M. and E.M.V. Milward–de-Azevedo'. 1998. Combined rearing of *Cochliomyia macellaria* (Fabr.), *Chrysomya megacephala* (Fabr.) and *Chrysomya albiceps* (Wied.) (Diptera: Calliphoridae) under laboratory conditions. J. Appl. Entomol. 122: 551–554.

Amendt, J., R. Krettek, and R. Zehner. 2004. Forensic entomology. Naturwissenschaften 91: 51–65.

Boatright, S. A., and J. K. Tomberlin. 2010. Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae). J. Med. Entomol. 47: 917–923.

Byrd, J. H., and J. F. Butler. 1996. Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. J. Med. Entomol. 33: 901–905.

Chaudhury, M. F., and L. A. Alvarez. 1999. A new starch-grafted gelling agent for screwworm (Diptera: Calliphoridae) larval diet. J. Econ. Entomol. 92: 1138–1141.

Chaudhury, M. F., and S. R. Skoda. 2007. A cellulose-based diet for screwworm (Diptera: Calliphoridae) larvae. J. Econ. Entomol. 100: 241–245.

Chaudhury, M. F., and S. R. Skoda. 2009. Diet pH and viscosity affect development and survival of screwworm larvae (Diptera: Calliphoridae). J. Econ. Entomol. 102: 799–803.

Chaudhury, M. F., L. A. Alvarez, and J. B. Welch. 1998. An alternative source of blood protein for screwworm (Diptera: Calliphoridae) larval diet. J. Econ. Entomol. 91: 1397–1400.

Chaudhury, M. F., L. A. Alvarez, and L. L. Velazquez. 2002. Recycled paper products as substitutes for gelling agent in screwworm (Diptera: Calliphoridae) larval diet. J. Econ. Entomol. 95: 1337–1341.

Chaudhury, M. F., S. R. Skoda, and A. Sagel. 2011. Solidifying agent and processing of blood used for the larval diet affect screwworm (Diptera: Calliphoridae) life-history parameters. J. Econ. Entomol. 104: 1103–1107.

Hall, D. G. 1948. The blowflies of North America. Thomas Say Foundation, Baltimore, MD.

Harris, R. L., R. D. Peterson, III, M. E. Vasquez–Guevara, and O. H. Graham. 1984. Gelled media for the production of screwworm larvae. Southwest. Entomol. 9: 257–262.

Harris, R. L., E. F. Gersabeck, C. Corso, and O. H. Graham. 1985. Screwworm larval production on gelled media. Southwest. Entomol. 10: 253–256.

Harrison, B. A., and W. G. Pearson. 1968. A case of aural myiasis caused by *Cochliomyia macellaria* (Fabricius). Mil. Med. 133: 484–488.

(NCSS) Number Cruncher Statistical System. 2000. NCSS statistical system for Windows, user's manual, version 6. NCSS, Kaysville, UT.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Freeman, New York, NY.

Taylor, D. B. 1988. Comparison of two gelling agents for screwworm (Diptera: Calliphoridae) larval diets. J. Econ. Entomol. 81: 1414–1419.

Taylor, D. B., and R. L. Mangan. 1987. Comparison of gelled and meat diets for rearing screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae) larvae. J. Econ. Entomol. 80: 427–432.

Taylor, D. B., J. C. Bruce, and R. Garcia. 1991. Gelled diet for screwworm (Diptera: Calliphoridae) mass production. J. Econ. Entomol. 84: 927–935.

Zumpt, F. 1965. Myiasis in man and animals in the old world. Butterworths, London, United Kingdom.

Received 31 August 2012; accepted 28 May 2013.