Report on *Hermetia illucens* large scale production

Deliverable 1.3

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Content

1. Rearing black soldier fly larvae

1.1. BSF life cycle

To rear black soldier fly larvae is to understand its life cycle and exploit its stages in order to maximise production. The life cycle is shown in Figure 1 and consists of the adult fly which will start laying eggs after two days of mating and two days of egg development. A neonate will hatch from the eggs four days later and it will grow into a black prepupae in at least another 12 days. The white larvae just before their last moulting are the most interesting stage for harvest. The prepupa has stopped eating and is looking for a quiet spot to harden into a pupa which can take up to 7 days. The pupae then takes another 6 to 10 days for a fly to eclose and start the cycle all over again.

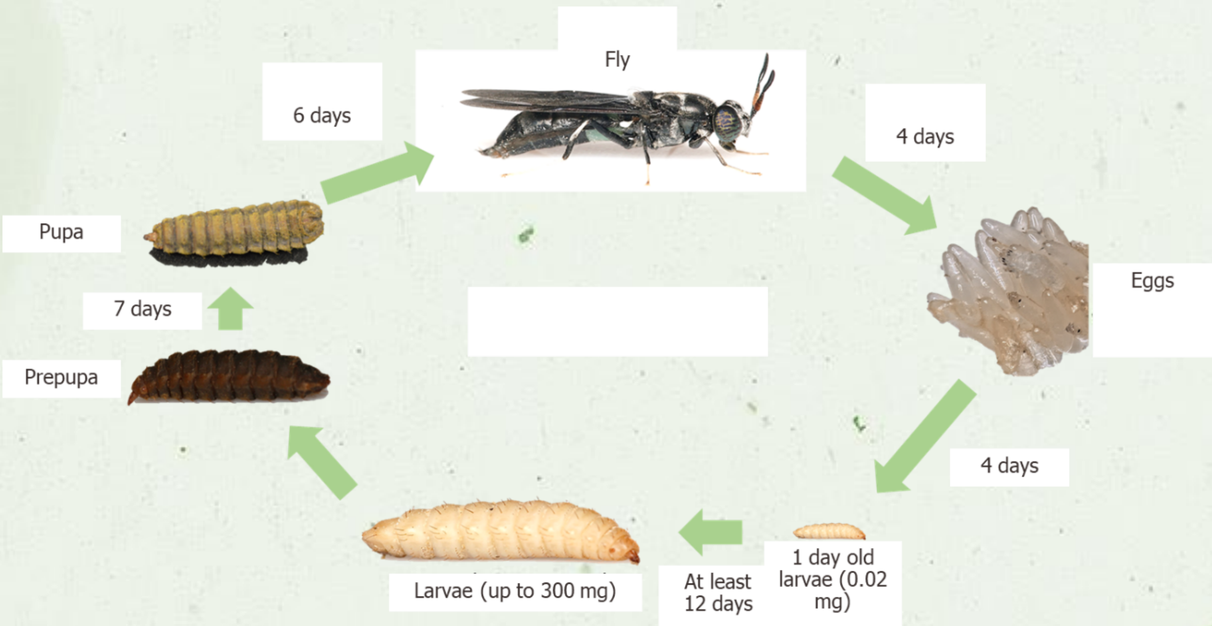

Figure 1: The life cycle of the black soldier fly.

1.2. Fly housing

Flies can be kept in different types of housing although most concepts can be boiled down to a few fundamentals. These fundamentals are:

- Climate conditions:

The black soldier fly is a tropical species and as such they thrive in tropical conditions. 30 °C and a high relative humidity of around 80% is recommended.

- Pupae storage:

Usually flies are loaded into a cage by letting pupae eclose adjacent to the cage. There they are stored without any direct lighting. When they eclose the flies are attracted by the light from the cage which guides them inside. This spatial separation of pupae and flies is intentional and quite recommendable. Pupae can still carry a specific smell and as explained later on, smell is used as a way to centralise oviposition. The presence of pupae in the cage could therefore potentially interfere with a controlled, centralised egg production.

- Light:

Light is crucial to have successful mating and oviposition. There is always the option to keep the flies in natural daylight. However, due to variation in local weather it is recommended to have at least artificial light as a backup or rear the flies fully in artificial light, especially in temperate climates. Bright cold light with a higher proportion of short wavelengths, i.e. blue light, is suitable as an artificial light source. At Inagro we use, LED high bay lights (100 W, 5700 K). However, keep in mind that some fly strains with no history of being reared in artificial light might have problems to do adapt, possibly due to a lack of UV light in standard artificial light. Knowing the background of your colony is therefore helpful to have successful mating and oviposition.

- Attractant and egg traps:

After mating, female flies will start looking for a suitable spot to lay their eggs. Flies like to lay their eggs in crevices. Any will do, so in order to have some control over the location where the flies will oviposit, it is recommended to use an attractant. This attractant is a smelly organic substance that triggers the instinct of the flies as they associate this smell with the presence of feed for their offspring. As such, the flies are inclined to lay their eggs close by. For example, at Inagro we use an attractant similar to what is proposed by Dortmans et al. (2021), which consists of a mixture of dead flies, frass and water. By providing an egg trap near this lure, most of the eggs can be collected at this centralised spot. As an egg trap stacked plates (preferably wood) with a 1 mm spacing will do (Figure 2).

Figure 2: A black soldier fly laying its eggs in an egg trap.

All of the above combined leads to the current fly cage that is used at Inagro (Figure 3). We opt to treat each cage as a batch and only insert pupae at one occasion, leave them to eclose, mate and oviposit, the flies will naturally perish within a few weeks. Afterwards the cage can be cleaned thoroughly and prepared for the insertion of a new pupae batch.

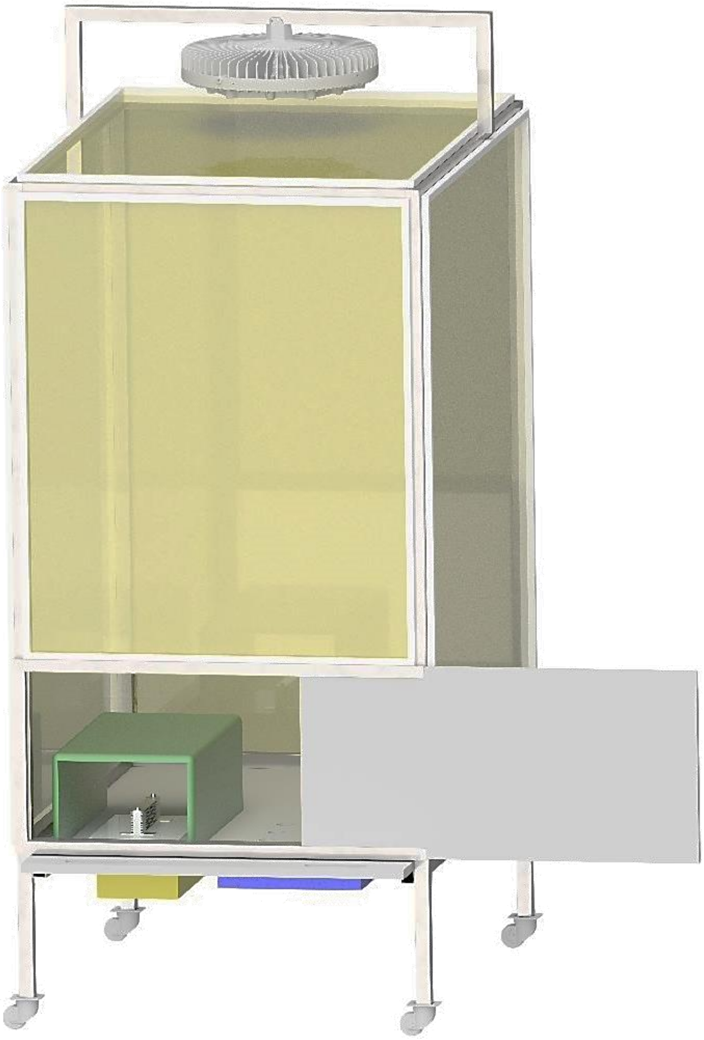

Figure 3: The cage used for egg production usesd at Inagro. The green part is a house with perforated bottom plate which the egg trap on top, below the green house hangs a box with attractant (in yellow). Below the bottom plate hangs a blue box, carrying the pupae that can enter the cage via openings in the bottom plate.

1.3. Egg production

Per cubic metre of cage we house 1 kg of pupae, the equivalent of 5 to 10 thousand flies, depending on the size of the pupae. Data has shown that smaller female flies lay fewer eggs than larger counterparts (Figure 4 (left)). A fly that is double the size will lay thrice the amount of eggs. We therefore recommend to rear larvae meant to reproduce and maintain the colony on a high quality feed in order to have large flies with a high reproductive capacity.

One batch of flies of a similar age will lay their eggs within a time frame of one week. From one cage filled with 1 kg of pupae egg yield will vary depending on the size of the flies and also on the quality of the pupae. The egg yield prediction for one kg of small pupae (100 mg) is estimated between 24 and 65 g (95 % confidence interval (CI)). For one kg of large pupae (200 mg) egg yield is expected between 49 and 90 g (95 % CI). The data and model on which this prediction is based, is shown in Figure 4 (right).

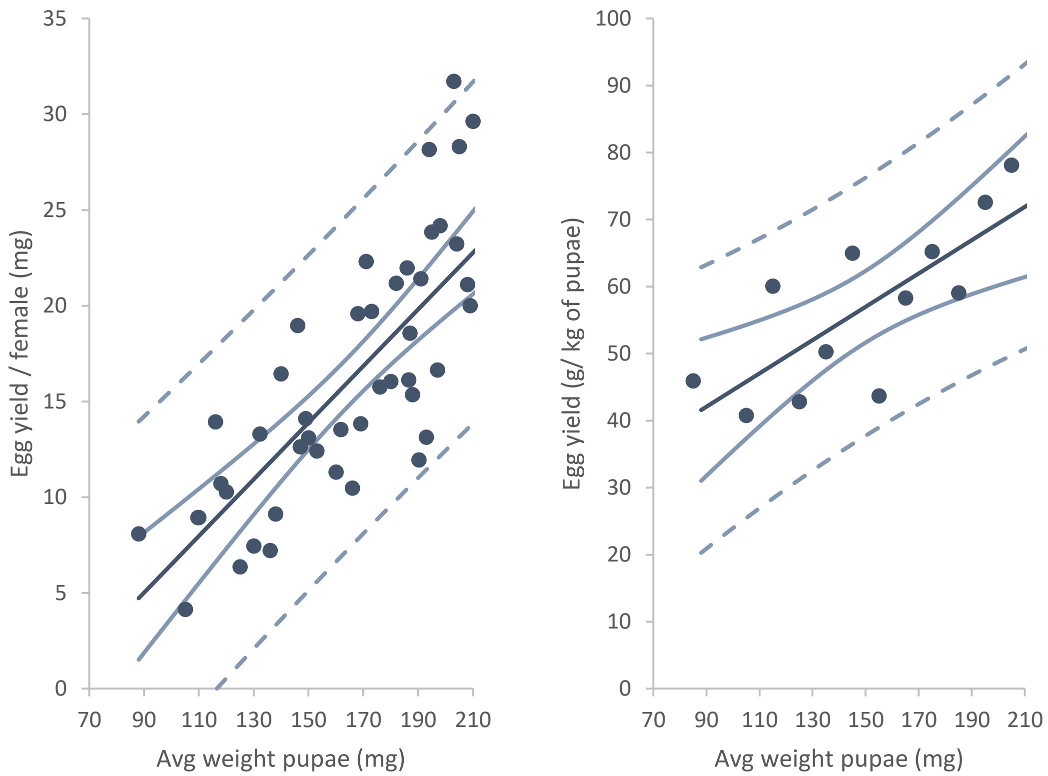

Figure 4: Egg production per female (left) and egg yield per cage with 1 kg of pupae (right).

1.4. Hatching eggs

Eggs are best hatched at 30 °C and 80% relative humidity. The eggs can be hatched above their starter feed, although this has same drawbacks as larvae that hatch a day later will have a delay in development compared to early hatchers. We recommend to hatch the eggs in a dedicated bucket as it allows to collect pure freshly hatched larvae (neonates). This will approximately be 50% of the amount of eggs (on a weight basis).

1.5. Raising neonates

The neonates are initially barely visible with the naked eye and they only weigh 0.02 mg. When the neonates are collected they are placed on a starter feed, at Inagro we use Gainesville diet. Gainesville diet is a mixture of water (67%), wheat bran (16.5%), corn meal (6.6%) and alfalfa (9.9%). Per rearing crate (60 x 40 cm) we add 5 kg of feed and 5 g of neonates, which are approximately 240,000 larvae. After 5 to 6 days all the feed is consumed and the larvae will weigh 2 to 3 mg.

1.6. Producing larvae for harvest

After the nursing phase the larvae are transferred to new crates with fresh feed for the second stage of raising the larvae. We add 15,000 to 20,000 larvae per 10 kg of fresh feed per rearing crate (60 x 40 cm). This setup is expected to yield 1 up to 2.5 kg of live larvae after a period of 7 to 14 days. The yield is strongly dependant on the quality of the feed. More details can be found in the combined report for deliverable 1.1 and 1.2 (Compound diets formulation and *Hermetia illucens* larvae performance). When all the feed is consumed, the frass (excrements of larvae, exuviae, dead insects and remaining feed) should be relatively dry and granular. If this is the case the frass can be easily sieved from the larvae.

1.7. Maintaining the population

Larvae used to maintain the population get a special treatment, they are kept on a high quality feed (in our case Farm 1 Crumble™) and a lower larval density. Larvae destined for production get 0.67 g of feed per larvae while larvae for reproduction get at least 0.8 g of feed per larvae. This method is expected to yield prepupae of 150 mg while maintaining the advantage of having dry frass that can be easily sieved.

2. Trouble shooting: storing BSF pupae

One of the main challenges of rearing black soldier fly larvae is getting a stable production with a predictable harvest at fixed intervals. Having the option to use a diapause in the lifecycle of the black soldier fly is one powerful tool to do so. Inducing a diapause allows to delay certain developmental phases, which makes it possible to store a certain life stage and activate it again when needed. This reassures that an insect farmer does not have to act ad hoc when a new life stage of the black soldier fly occurs. For example, when a new batch of pupae is on the verge of eclosing but the insect farmer does not need any flies at that moment, for instance when all his cages are still producing eggs. In that case it would be useful to pause pupae development until new flies are desired. Luckily, insects are ectotherms, which means temperature can be used to tweak their development time as desired.

With this in mind, a trial was performed in which black soldier fly pupae were stored at 15 and 18°C in order to delay and potentially halt pupae development.

2.1. Material and methods

For this trial black soldier fly pupae were collected from the standard population from Inagro. These larvae were reared under standard rearing conditions for larvae meant for reproduction.

A batch of prepupae was checked on a daily basis. When pupation had set in they were transferred to a wine cooler set at the experimental temperature (15 °C or 18 °C). The pupae were stored per 5 in a plastic container, this served as the experimental unit. Different treatments were stored in the cooler for different amounts of time: 0, 2, 4, 7, 14, 21, 28 and 35 days. After each period 4 experimental units were removed from the cooler and placed back at 30 °C. The pupae were checked daily and the number of eclosed flies was monitored.

2.2. Results and discussion

The data is a time series with values ranging from 0 up to 5 flies. A dose-response curve was fit on the data, with the dose being the pupae age (Figure 5). The time for a treatment to reach 50% eclosion was estimated and the correlation between the time in the cooler and the time before eclosion was plot. A linear model was fit on the data for both temperatures (Figure 6).

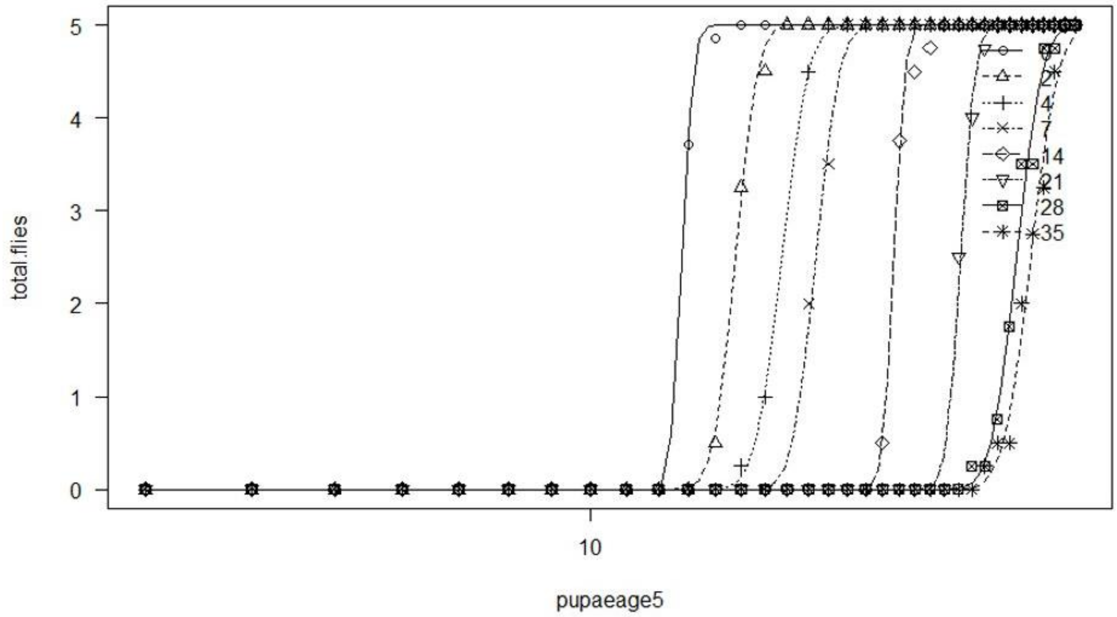

Figure 5: The time (days) since pupation and the amount of flies that have eclosed for different times (days) in the cooler on a log scale (at 18 °C) with a dose response curve fit on the data.

For both experiments we see that pupae that are kept at 30 °C will take 8 to 9 days before 50 % of them will have eclosed. The develoment of the pupae is delayed differently depending on the temperature they were stored. At none of the two tested temperatures a true pause of development was observed. However, there is a delayed development in both cases. When the pupae are stored at 15 °C development is reduced to only 15% of what is observed at 30 °C. At 18 °C development is at 31% of its “normal” development rate.

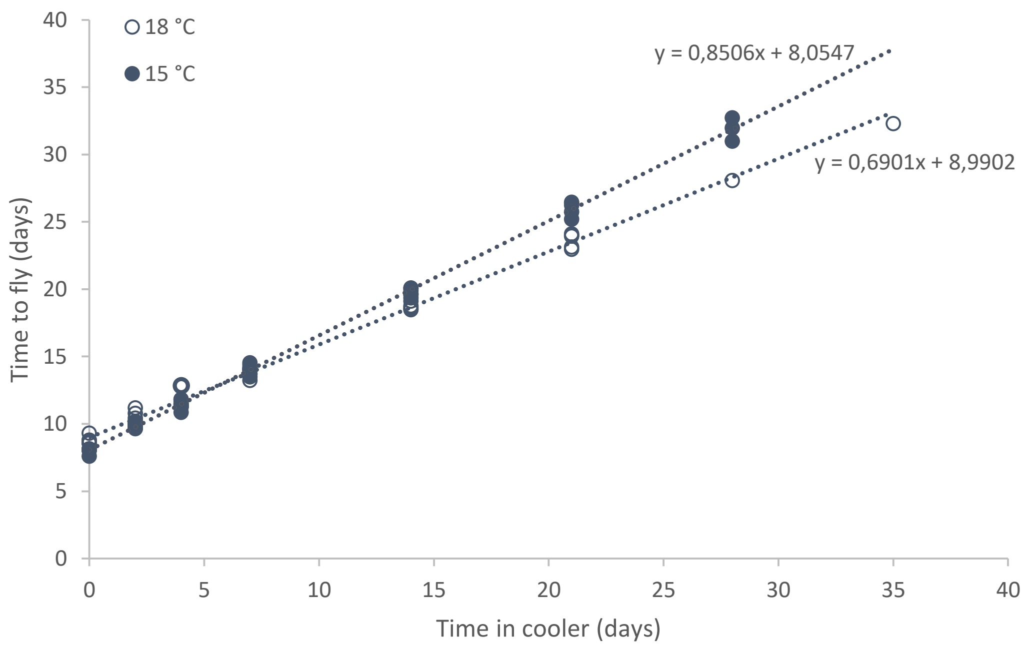

Figure 6: The time the pupae spend in the cooler in function of the time until 50% of the flies have eclosed.

Storing at lower temperatures comes with some risks. First of all, mortality remarkably increased when pupae were stored longer than 14 days at 15 °C, as shown in Figure 7. No such adverse mortality effects were observed at 18 °C

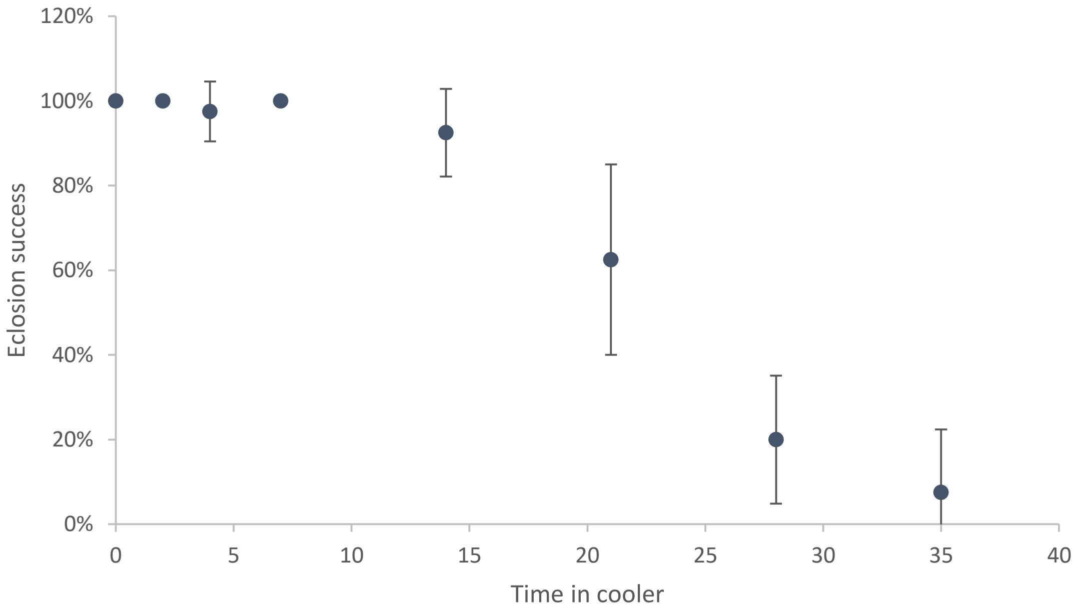

Figure 7: The number of successful eclosions in function of the time pupae were stored in a cooler at 15 °C.

2.3. Conclusion

50 % of the pupae will develop within 8 to 9 days into flies when they are left to develop at 30 °C. This can be delayed by storing pupae at lower temperatures, one day at 30 °C equals to 6.7 days when cooled at 15 °C and 3.2 days at 18 °C. The time that the larvae can be stored is limited as they will eventually start to eclose in the cooling (at 18 °C) or mortality will drastically increase (at 15 °C). A general recommendation would be to store fresh pupae no longer than 14 days in the cooler in order to preserve quality but allowing for some flexibility on when to start a new fly cage.

3. Black soldier fly larvae farming on a poultry farm

The question remains how large of a black soldier fly farm is needed to produce a sufficient amount of larvae to supply an organic poultry farm. To answer this question, a specific case will be studied. As a model organism for medium growing organic poultry, the Label Rouge Naked Neck was chosen. The same genotype that was tested during the first feeding trial of Poultrynsect. In that trial a 10 % supplementation of the daily feed intake was maintained. The daily feed intake increases with the size of the birds, the weekly requirements are shown in Table 1. Assuming that an organic poultry farm houses up to 10,000 animals with an all in, all out system, at its peak a total of 920 kg of live larvae will need to be produced.

Table 1: Weekly feed intake of live larvae assuming 10% supplementation.

Week	Live BSF larvae (g/week/chicken)
1	26.3
2	44.9
3	53.3
4	60.7
5	67.6
6	74.2
7	80.2
8	86.1
9	92

The production flow and the associated output one would manage to get from 1 kg of pupae is shown in Figure 8. A conservative output from 1 kg of pupae would be 135.8 kg of live larvae, assuming a feed conversion of 2 kg of dry feed per kg of live larvae, which is to be expected on a low quality feed composed of sidestreams. When applying this to the desired 920 kg live larvae required for a farm with 10,000 birds, 7 fly cages will be needed during the one week of peak demand. For 920 kg of larvae a total of 6140 kg of larvae feed divided over 614 crates is needed during the final rearing stage. This rearing size requires two rooms of approximately 8 by 6 metres. One room to house the flies and produce the eggs and one room to rear the larvae. It is assumed that the crates are stacked 10 high.

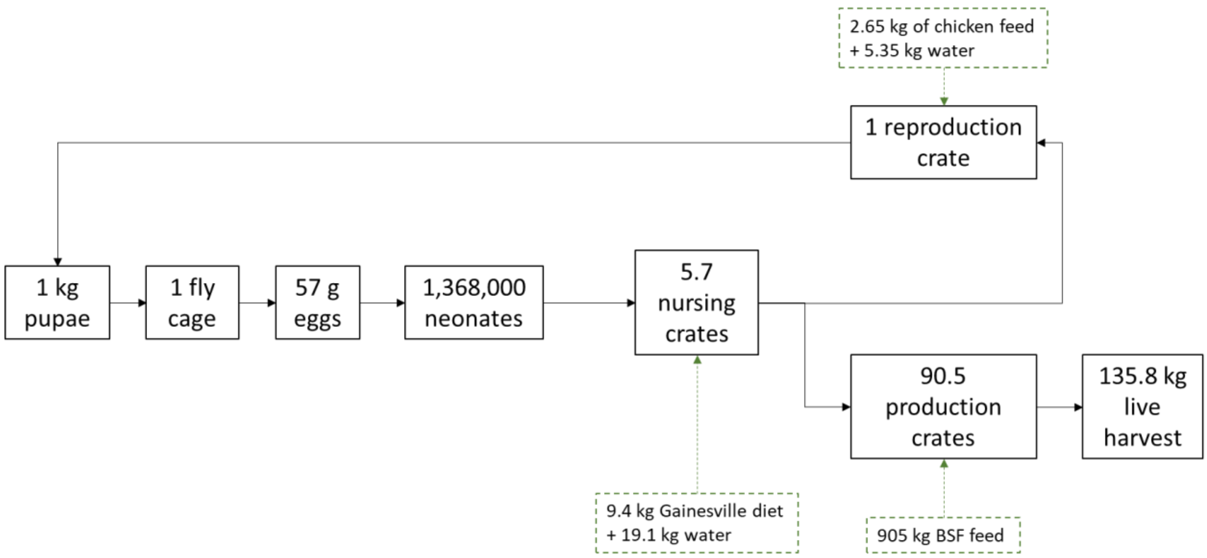

Figure 8: The production flow of 1 kg of pupae and required feed amounts.

References

References

Dortmans B.M.A., Egger J., Diener S., Zurbrügg C. (2021). Black Soldier Fly Biowaste Processing - A Step-by-Step Guide, 2nd Edition. Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland.

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