



The PRIMA programme is supported under Horizon 2020 the European Union's Framework Programme for Research and Innovation



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# SUSTAVIANFEED

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ALTERNATIVE ANIMAL FEEDS  
IN MEDITERRANEAN POULTRY  
BREEDS  
TO OBTAIN SUSTAINABLE  
PRODUCTS

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POTENTIAL FOR INSECT  
PRODUCTION IN EACH  
PILOT

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DELIVERABLE 2.2

*This project (grant Number 2015), is part of the PRIMA programme, supported by the European Union*

Partners



Slow Food Foundation  
for Biodiversity



PROJECT INFORMATION	
Type of Project	PRIMA Foundation Project
Topic	Topic 1.2.1-2020 (RIA/IA) Genetic conservation and animal feeds: Sub-topic B) Alternative animal feeds (IA) of the Annual Work Plan 2020 for the Partnership for Research and Innovation in the Mediterranean Area (PRIMA)
Grant Agreement No.	2015
Project Duration	01/04/2021 – 31/03/2025 (48 months)
Project Coordinator	SOCIEDAD AGRARIA DE TRANSFORMACION 2439 (ALIA)
Project Partners	UNIVERSITY OF MURCIA (UMU), UNIVERSITY OF TURIN (UNITO), INSTITUT SUPERIEUR AGRONOMIQUE DE CHOTT MARIEM (ISA-CM), ASSOCIATION RAYHANA (RAYHANA), ENTOMO CONSULTING S.L. (ENTOMO), EGE UNIVERSITY (EGE), FONDAZIONE SLOW FOOD PER LA BIODIVERSITA' ONLUS (SLOWFOOD)

DOCUMENT INFORMATION	
Title	POTENTIAL FOR INSECT PRODUCTION IN EACH PILOT
Version	1
Release Date (dd.mm.yy)	29.04.22
Work Package	WP2
Dissemination Level	PU

DOCUMENT AUTHORS AND AUTHORISATION	
Document Responsible	ENTOMO
Contributors	ALL
Reviewed by	ALIA, UNITO and UMU

DOCUMENT AUTHORS AND AUTHORISATION	
Approved by	ALIA

DOCUMENT HISTORY			
Version	Date (dd.mm.yy)	Description	Implemented by
1.00	11.04.22	First draft for revision and suggestions	ENTOMO
1.01	19.04.22	Revised first draft	ALIA, UMU and UNITO
2.00	25.05.22	Second draft with suggestions	ENTOMO
2.01	26.04.22	Second version of the deliverable with the inputs from the review	ALIA
3.00	27.04.22	Final version	ENTOMO

## SUMMARY

The main objective of the deliverable is to report on the preliminary study of the Insect Pilot installation in the different locations. The aim of the document is to create a piece of base information or such as a handbook for the partners with no previous experience in insect farming, in other to get knowledge in terms, actions, and requirements needed. The document is associated with task 2.2. Analysis of insects' farming in each territory. It intends to be a base for the execution of task 3.1. Implementation of insects' farms at local sites. The document starts with a bibliography review covering aspects of logic of the insect circular economy approach and biology, continues remarking on the requirements and activities necessary for the insect production, makes a summarised description of the different locations, and finalise with a model Insect Pilot proposition and planning which will be executed during Task 3.1.

## TABLE OF CONTENTS

<b>SUMMARY .....</b>	<b>4</b>
<b>TABLE OF CONTENTS .....</b>	<b>5</b>
<b>Acronyms and abbreviations .....</b>	<b>7</b>
<b>Table of Figures .....</b>	<b>8</b>
<b>List of tables .....</b>	<b>8</b>
<b>1 INTRODUCTION .....</b>	<b>9</b>
<b>2 LITERATURE REVIEW OF THE BIOLOGY AND LIFE CYCLE .....</b>	<b>11</b>
2.1 Rational of the approach .....	11
2.2 Main species used .....	14
2.3 The role of the species .....	14
2.4 Biology and Life cycle .....	15
<b>3 TECHNICAL REQUIRMENTS AND ACTIVITIES .....</b>	<b>20</b>
3.1 Premises .....	20
3.2 Breeding .....	21
3.3 Nursery .....	22
3.4 Fattening .....	22
3.5 Gathering and feeding .....	22
3.6 Pre-pupa development .....	22
3.7 Pupa development .....	23
3.8 Quality control .....	23
3.9 Machinery .....	23
3.10 Risk Management .....	24
<b>4 LOCATIONS CHARACTERISTICS .....</b>	<b>29</b>
4.1 Generalities in weather and insect production .....	29
4.2 Turkey .....	31
4.3 Tunisia .....	31
4.4 Italy .....	32
4.5 Spain .....	33
<b>5 GENERAL DESIGN PROPOSITION AND ACTION PLAN .....</b>	<b>34</b>
5.1 Big scale (Spain) .....	34
5.2 Medium Pilot scale (Tunisia) .....	36
5.3 Small farm/household scale (Tunisia) .....	39
5.4 Planning .....	40
<b>6 CONCLUSIONS .....</b>	<b>43</b>
<b>REFERENCES .....</b>	<b>44</b>
<b>ANNEX I. Data collected from the partners .....</b>	<b>48</b>
1.1 Turkey .....	48
1.2 Tunisia .....	50
1.3 Italy .....	52
1.4 Spain .....	53



## Acronyms and abbreviations

Abbreviation	Description
BSF	Black Soldier Fly
HVAC	Humidity Ventilation and Air Conditioning
IPP	Insect Production Pilot
OECD	Organisation for Economic Co-operation and Development
QC	Quality Control
RH	Relative humidity
SDGs	Sustainable Development Goals

## Table of Figures

Figure 1. Different food sources considered by Parodi <i>et al.</i> (2018) as human diet alternatives for more sustainable food production. ....	13
Figure 2. Larval stage (left) and adult (right) of <i>H. illucens</i> .....	15
Figure 3. Sexual dimorphism between males (M) and females (F) in <i>H. illucens</i> .....	16
Figure 4. Life cycle of <i>H. illucens</i> .....	17
Figure 5. Growth in mass (mg) of <i>Hermetia illucens</i> larvae measured daily (mean $\pm$ SD). Larval stadia 1–7 are delimited by vertical dotted lines based on statistical analysis of head capsule widths. (Adapted from Gligorescu <i>et al.</i> , 2019).....	19
Figure 6. BSF Attributes.....	19
Figure 7. Production cycle and activities division .....	21
Figure 8. Graphic showing the temperature conditions (maximum, average and minimum) in Murcia city, along the Mediterranean basin in Spain year of 2019. Data extracted from CARM.es	30
Figure 9. The ENTOMO's IPP to be used during the project execution. Up, General view in 3D of the IPP; down left internal corridor connecting with the chambers and; downright the production structure.....	36
Figure 10. Typology of IPP to be constructed and installed in ISA-CM: Up-left, external view of the container where the walls act as illustration for the project iconography; Down-left, interior part of the container showing fattening area and; Right, breeding ca .....	38
Figure 11. Inside view of the container: Left area for feed preparation and larvae extracting and cleaning; Right, laboratory for QC activities and assessment. ....	39
Figure 12. Inside view of the container: Left area for feed preparation and larvae extracting and cleaning; Right, laboratory for QC activities and assessment. ....	39
Figure 13. Gantt for IPP activity .....	40
Figure 14. Summary of IPP characteristics .....	41

## List of tables

Table 1. Machinery and equipment used in the production phases .....	23
Table 2. Risk description and its management in insect operations. ....	25
Table 3. Space distribution used in each production area .....	34
Table 4. Space distribution used in each production area Tunisia IPP .....	36
Table 5. Planning of the IPP .....	41
Table 6. Planning for dry larvae supply to the feeding trials .....	42



## 1 INTRODUCTION

ENTOMO is a company dedicated to offering services on the design of facilities for the industrial production of Black Soldier Fly (BSF) mainly by transforming underutilised materials (waste or by-products) into higher valuable products. The main role in the SUSTAVianFEED project is to create Insect Production PILOTS (IPP) to integrate with the poultry production Pilots and to produce the insects the larvae biomass used during the poultry feeding trials.

In this project, part of the current feed ingredients will be substituted for alternative ones. However, those ingredients are mainly vegetable sources and have some limitations at the time in the formulation of feeds. At that point, the use of insects as a way to supply nutrients has some interest. On one hand, insect larvae are a natural diet in chicken and its use could give extra properties to the feed, affecting positively to poultry health by improving welfare or providing substances by the larvae (chitin, enzymes or functional oils). On the other hand, the insect larvae offer a rather stable composition (protein, fats, minerals and moist) compared with the by-products used in the larvae feeding, with different components levels, fibre and water content. In this project larvae will be fed with different by-products and raised from 8 mg larva to 250 mg larva in a period (12 days) and harvested and processed to obtain mainly dry larvae (with about 45% protein, 35% fats, 10% chitin and 10% ashes) to serve as one of the ingredients to feed the poultry in the Pilots.

Although insects are becoming more relevant to produce proteins while feeding them with organic by-products or waste, and some information is starting to appear about how a production facility should be, and about how to produce them in a safe way and meeting with quality standards and regulations. However, little information is available about how this circular economy approach (waste-insect-poultry) could really profit poultry production and local economies either at large or small scale, as well as understanding how could be integrated and at which scale in different areas.

Along this project, the potential of using insects as feed ingredient for the production of chickens will be explored. And the insects will be produced sustainably by feeding on agriculture waste/by-products materials, focussing on those feedstuffs that cannot be used directly with chickens. Thus, the role of the insects will be to recover the nutrients from those waste materials, concentrate and converted them into animal protein (insect biomass) which can be fed to poultry and an easy and concentrated form.

The main goal of the activity is to give the specifications for the design of 3 different facilities able to produce larvae at different locations and to record the data to be used later on for different business models implying insect's production for animal feed in the different regions. As part of the project works, it has been envisaging the use of 3 IPP. One IPP will be operated in Murcia, as a representation of high controlled environment, where the larvae will be produced to provide material for the feeding experiments of UMU and ALIA. Another IPP will be constructed by ENTOMO and sent to Tunisia to be installed at ISA-CM. This IPP will serve as an entomological lab for testing insect feeding with different substrates and as a training point for students or Tunisia entrepreneurs in insect production. In addition to that a third IPP will be supplied to Rayhana as representation of low tech IPP easy to implement at household and small farm levels. These 3 IPP aim to encourage and promote the SUSTAVianFEED approach at the 3 levels, high, medium and

small-scale waste-insect-poultry circular economy approach. Besides these IPP operation other activities will be done with UNITO and EGE. In EGE, interactions have been created and will further develop with local insect producers in other to have similar insect productions standards in all the pilots. With UNITO, larvae will be produced and supplied by ENTOMO to carry out the poultry feeding experiment and interactions with other actors will be implemented to facilitate similar larvae quality and to maximise experiment impact.

The deliverable focus in setting the base in terms of IPP for the rest of the project. The document is structured in several sections. Section 2, covers a bibliography review with rational of the approach, the role of the species, biology, live cycle of the insect. Section 3, using as a base the biological knowledge, describes the activities and requirements to carry out the insect production, such as breeding, nursery, fattening, processing and risk management. Section 4, shows the results of studying the different locations and its potential for the IPP. And in Section 5, there is a design proposition for the different IPP foresing in this project. The information in the deliverable will serve not only as information and description but also as part of the information necessary to implement the pilots in the different locations, since the administrations normally require a dossier with information in order to provide permission for the activity.

This deliverable will be reviewed during IPP implementation to include the newest additions to the IPP and to complete the initial information available.

## 2 LITERATURE REVIEW OF THE BIOLOGY AND LIFE CYCLE

To set the base of the insect production, the first part of the process is to know and understand what are the characteristics of the specie. Then, the information can be used to plan the production, predict complications and help to solve future problems. This section it is shown the literature review of BSF covering the aspects of rational of the approach, the role of the species and the biology.

### 2.1 Rational of the approach

Changing industrial and commercial processes towards a more circular and sustainable approach is a key challenge of the current century. The ongoing awareness of the importance of re-design the economic mechanisms is crucial to defining the present and future general wellbeing. The scarcity of natural resources invites scientists, entrepreneurs and all society to review consumption and, especially, the role in waste of industries, agriculture and cities.

According to the United Nations World Population Prospects (2019), the world's population continues to grow, from the current 7.7 billion to reach around 8.5 billion in 2030, 9.7 billion in 2050, and 10.9 billion in 2100. Moreover, living standards are projected to increase worldwide, and gradually converge towards those in the most advanced countries (OECD, 2019). The depletion of natural resources is obvious and not enough sources are available for the whole population. It is urgent the need of optimizing all processes involving natural resources use, in special, tackling what is still considered waste and turning it into a productive resource in another process.

Solid waste management is an essential aspect of planning sustainable and human-orientated smart cities, but it is still often overlooked. Even though some improvements and innovations in the field have been popping up worldwide, waste management can be the most expensive item in municipal budgets, which explains its precariousness, being frequently associated as a driver of inequality and environmental degradation.

According to the What a Waste report (World Bank, 2018), about three-quarters of waste in Europe and Central Asia could be potentially recovered through recycling or organics management. However, only about 31 percent of waste materials are in fact being recovered through recycling and composting.

The turning point of this archaic, expensive and ineffective model is the development of innovative technologies that redefine the value of "waste", converting it into an asset of great value in the so-called Circular Economy.

While much progress has been made in the economic valorization of the dry fraction of municipal solid waste, such as plastic, paper and glass, the organic fraction has remained mostly stuck in obsolete and costly to maintain models such as Composting Plants, or in the exploitation of its minimum energetic value (methane burning or compost) or even completely neglected, as in some developing countries.

The waste composition differs substantially according to the income level. The organic matter share of waste goes down as the wealth level rises (World Bank, 2018). In this context, food and green waste account for more than 50% of waste in low- and middle-

income countries. While in high income countries, due to higher amounts of packaging residues and other non-organic wastes, the organic matter fraction is around 32% in absolute terms. However, the richer countries produce more waste per capita, which in absolute terms shows an undeniable urgency in the development of attractive technologies for managing the huge amounts of organic waste produced every day.

Solid waste management has evolved considerably from the early practices of open dumping and burning to more controlled but analogous methods such as landfilling and incineration. Even though practices like reduction, reuse, recycling and composting have grown in importance and technology in recent years, the truth is that landfill remains the most usual final disposal option for non-hazardous, non-industrial waste, as municipal solid waste worldwide.

Landfills release leachate and gases, emit unpleasant odours, carbon emissions, and attract pests. Moreover, landfills are running out of capacity and closing, reducing the space available for future waste and requiring further landfill site development. Sustainable approaches to waste management can minimize the burden on landfills and lead to the production of valuable materials, following the Circular Economy approach, such as compost and animal feed. The rearing of BSF is one of these potential solutions.

Therefore, to reduce land-use and deforestation pressures, while setting the ground to achieve the Sustainable Development Goals (SDGs) such as; 2 zero hunger; 8 decent work and economy growth; 11 sustainable cities and communities; 12 responsible consumption and production, It will be necessary to make changes in the ways of producing and consuming food, especially in relation to meat obtained from animals whose balance in the use of resources is unfavourable - such as beef. According to Parodi *et al.* (2018), altering the human diet is the key to success, suggesting the inclusion of novel food sources in a nutritional balanced diet, as stated in the following graphic (Figure 1):

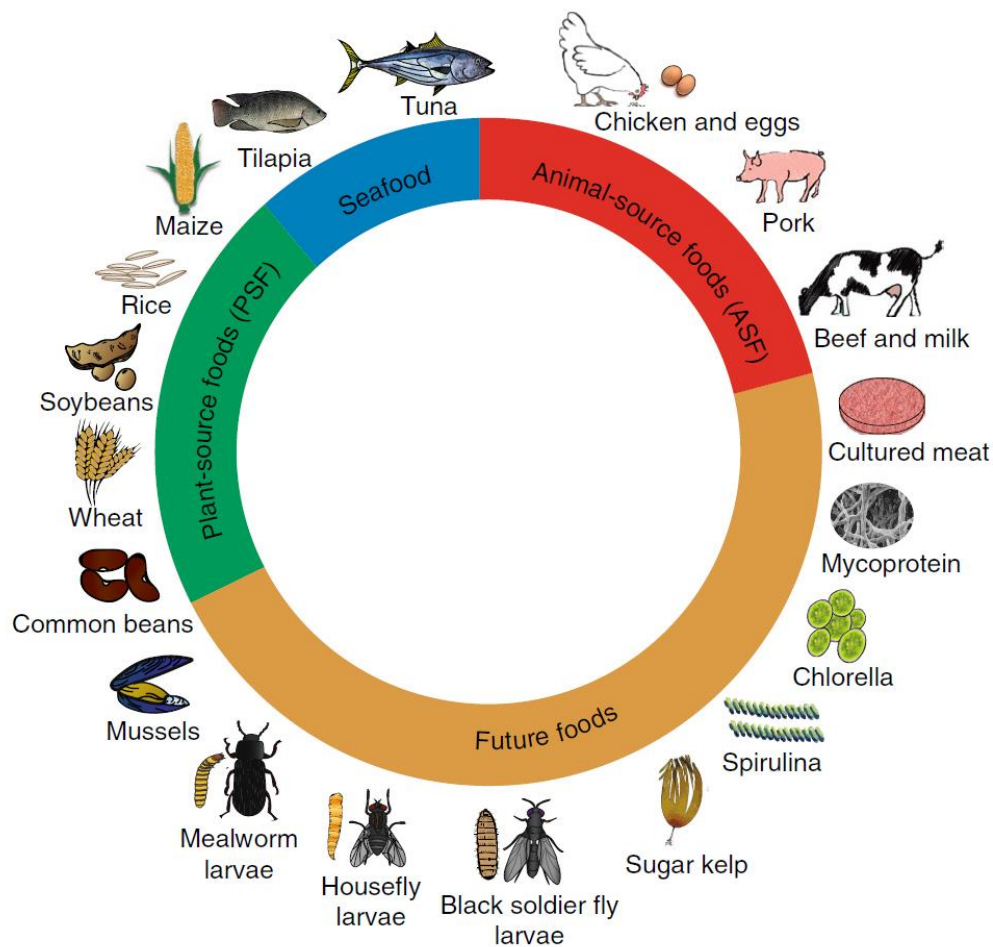


Figure 1. Different food sources considered by Parodi *et al.* (2018) as human diet alternatives for more sustainable food production.

From the Life Cycle Assessment (LCA), Parodi *et al.* (2018) found that from a mixture of future foods it is possible to provide all the essential macro and micronutrients for the human diet. For example, calcium, mainly obtained from milk today, can be replaced by Sugar Kelp and Black Soldier Fly larvae; iron, mainly obtained from red meat and eggs, is also found in most future foods, especially in chlorella and spirulina; Vitamin B12, absent in most plant-sourced foods, and commonly advised to vegans to take supplements to avoid health risks, is, however, abundant in future aquafeeds (Sugar Kelp, Chlorella and Spirulina) and in Black Soldier Fly larvae.

SUSTAvianFEED project comes as an attempt to combine current circumstances: (1) the need to increase economically attractive opportunities in rural areas; (2) the need for diversified means to produce livestock feed source in a more efficient way; (3) the need for innovative and profitable solutions to deal with the Municipal Solid Waste and cope to the European Waste Policy milestones. The project, with the use of BSF, will explore the implementation of insect breeding in several conditions along the Mediterranean area with the focus on verifying how further BSF breeding can be explored as a side activity in farm systems, or even urban areas, in Mediterranean countries.

## 2.2 Main species used

The main insect species used in this project is *Hermetia illucens* (Linnaeus, 1758), an insect belonging to the order of the dipterans. *H. illucens* (Linnaeus, 1758) (Figure 1) is commonly known as "soldier fly" also known as Black Soldier Fly and belongs to the Stratiomyidae family. This species is possibly native to America (Kovac & Rozkosny, 1995) but because of the anthropic activity, it has been distributed throughout all the humid and subtropical tropical regions of the planet (James, 1935). In Europe, this species was first recorded in Malta in 1926, and since then it has been cited in other countries of the Mediterranean area, such as Albania, Croatia, France, Italy, southern Switzerland, Portugal and Spain (Martínez-Sánchez *et al.*, 2011). In the Iberian Peninsula, *H. illucens* was registered for the first time in 1954 in Spain, and in Portugal in 1995 (Martínez-Sánchez *et al.* 2011). Although considered native to America, Benelli *et al.*, 2014 gave a more interesting citation of the species' presence in Europe by proving its presence as early (1470–1524) in the sarcophagus of Isabella d'Aragona, demonstrating the rather early importation by Spaniards soon after the discovery of America or the presence in Europe before taxonomist described it.

## 2.3 The role of the species

BSF larvae can consume a broad selection of organic material, from fruits and vegetables to animal carcasses and manure (Nguyen *et al.*, 2015). The BSF occurs naturally in a very wide range worldwide, in all regions between the latitudes of 40°S and 45°N (Dortmans, 2017), with the real possibilities of increasing habitat areas due to climate change effects. Since the adult BSF do not feed, the larvae need to accumulate a substantial fat body during the larval development for adult survival. The species has a main role in nature as saprophagous.

Unlike many saprophagous pests, BSF larvae do not carry bacteria or disease and are even capable of inactivating *Escherichia coli* and *Salmonella* spp. (Erickson *et al.*, 2004 apud Nguyen *et al.*, 2015). BSF larvae have some competitive advantages in relation to the housefly (*Musca domestica*) larvae, by the fact that BSF make the organic matter more liquefied which is undesirable to the housefly (Makkar *et al.*, 2014), and being a species with much better competitive abilities in terms of feed source dominance, particularly during the last instars of larval development. Furthermore, by restraining bacterial growth and by aerating and drying the organic matter through its natural behaviour, BSF larvae can reduce significantly the bad odour (Makkar *et al.* 2014). Additionally, the adult flies do not enter into houses and have a short lifespan, making them not considered pests (Tomberlin *et al.*, 2002a and Nguyen *et al.*, 2015).

The BSF became a revolutionary insect species due to its capacity to convert any organic waste and manure into high protein (40-44%) and lipid (7-36%) content providing a sort of inputs to the industry, especially as an alternative meal source for feeding livestock. Moreover, high quality chitin can be obtained from the exoskeleton of defatted BSF, which is also one of the fastest growing market in biopolymers. This chitin can be transformed into high-quality chitosan, which has potential applications in agriculture, veterinary, pest control, water treatment, food preservation and textiles, among others.

BSF is seen as a great opportunity to provide sustainable and economically profitable solutions to current worldwide food waste management problems (estimated at 1.3 billion ton/year: FAO, U.S.). The global BSF industry is expected to increase at a compound annual growth rate of 33.3% from 2019 to 2030 reaching a market value of 2,568.4 million by 2030 (Meticulous Research, 2020).

## 2.4 Biology and Life cycle

The larvae of this species have an essentially elongated shape (0.85 to 30 mm) with a tapered anterior end, and a rounded posterior. The tegument is whitish to darker, and strongly sclerotized according to age (Müller, 1925). Larvae can develop in a wide diversity of organic matter, from manure and decomposing meat, sludges to fruits and vegetables.

The adults (Figure 2) have a mimicry with certain groups of hymenoptera, which in principle gives them certain advantages over their predators. The female usually has a size larger than the male, although there is no obvious sexual dimorphism with the naked eye (Figure 3). The male genitalia is relatively short, and has two pairs of lateral posterior lobes, a pair of fences and a pair of very small gonostils. The sexual organ complex is very thin and dilated in its basal part (Gobbi, 2012). The female genitalia is composed of a pair of long fences formed by two segments; it has a long subgenital plaque in its distal part pointedly and a subtriangular genital furca (Üstüner *et al.*, 2003). The genital structure represents the only character of sexual dimorphism of this species. They cross six larval stages, and in their last pupa phase, they sclerotize and adopt a dark colour, until they form a black and immobile pupa (Figure 4).



Figure 2. Larval stage (left) and adult (right) of *H. illucens*

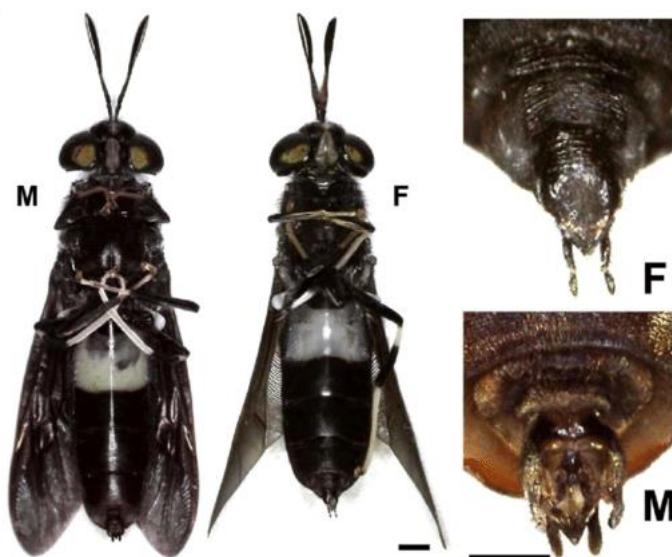


Figure 3. Sexual dimorphism between males (M) and females (F) in *H. illucens*

Adults have predominantly dark pigmentation, with brown or black wings. The size of the antennae is at least twice the length of the head, and they consist of eight irregular artefacts, the last of which is flagelomer, and has an edge. The legs are mainly black, although in the basal area of all tarsi a white pigmentation is observed. The abdomen consists of five visible segments of black colour, but at the back of the margin of tergites 1 and 2 there are a pair of translucent, white and oblong spots (Üstüner *et al.*, 2003).

As for the biological parameters of *H. illucens*, adults begin intercourse about 5 days after the emerging, and it takes place during the flight. After mating the females lay around 600 eggs (united in a cluster) in cracks or crevices near decomposing organic matter (Sheppard, 1983). Each oval-shaped egg measures approximately 1 mm in length, with a coloration that varies from white to pale yellow or cream. First stage larvae emerge at four days at 24 ° C (Booth & Sheppard, 1984).

The larvae can measure up to 3 cm in length in their last stage, they are dull and whitish in colour, and have a characteristic quetotaxia both in their ventral and dorsal part (Hall & Gerhardt, 2002). Once they emerge, the larvae begin to feed on organic matter, reaching an average reduction of 55% (Myers *et al.*, 2008; Newton *et al.*, 1995; Sheppard, 1983). Due to the high densities in which they develop, and the voracious appetite of the larva, fresh organic matter is processed extremely quickly, further suppressing bacterial growth or retaining it. In this way the bad smell due to the decomposition by the action of the microorganisms is minimized. This feeding activity is also capable of repelling the oviposition of housefly females (Bradley & Sheppard, 1984).

At the end of their growth, in the last larval stage (prepupa), the larvae leave the development medium looking for a dry and protected site. This stage is characterized by the hardening and darkening of the cuticle, as well as its great mobility. After a few days it becomes a pupa, which is characterized by the lack of movement (Hall & Gerhardt, 2002). The life cycle is schematized in Figure 4.



Adults emerge about two weeks after the formation of the prepupa (Tomberlin *et al.*, 2002). Adults do not need to feed, since they depend on the accumulated reserves during the larval phase (Newton *et al.*, 2005). This implies that adults never meet any rest of fresh or decomposing organic matter (Leclercq, 1997; Schremmer, 1986). Some studies indicate that certain light characteristics stimulate the mating of adults, it has been proposed that *H. illucens* eyes have unique characteristics of photoreception (Tomberlin & Sheppard, 2002; Zhang *et al.*, 2010).



Figure 4. Life cycle of *H. illucens*

The duration of each stage of the life cycle of *H. illucens* is influenced by various abiotic and biotic factors, which can significantly alter the development of the preimaginal stages of this species (Tomberlin & Sheppard, 2002). The optimum temperatures for the biological cycle of *H. illucens* are in the range 24 to 29.3 °C (Furman *et al.*, 1959). Ambient humidity can have important physiological effects affecting the development, longevity and oviposition of *H. illucens* (Gullan & Cranston, 2000). The optimum range for the development of the species is 50 to 99% relative humidity (Furman *et al.*, 1959; Tingle *et al.*, 1975; Bradley & Sheppard, 1983; Booth & Sheppard, 1984).

Other factors such as food quality and quantity as well as population density are of vital importance in the development of this species (Sheppard *et al.*, 2002; Tomberling & Sheppard, 2002). According to Liu *et al.* (2008) the amount of daily food that larvae require for their adequate growth depends on their nutritional content (Sheppard *et al.*, 2002). Ideally, the larvae take two weeks to reach the prepupa stage, but if there are food limitations, this period can be extended up to four months (Furman *et al.*, 1959). This ability to extend the larval state in response to food availability increases the chances of long-term survival under natural conditions (Sheppard *et al.*, 1994), allowing its adaptation to different types of habitats and means of development.

During the larval stage, from larval seeding (5-7 days old) to pupal formation, larvae can be exposed to unfavourable climatic conditions. Authors suggest that long exposure to 16°C might lead to the death of BSF at any stage of its cycle (Holmes *et al.*, 2016). However, the experience in the lab shows that only very low, close to zero, and very high temperatures (above 40°C) (Chia *et al.*, 2018), will result in immediate larval mortality and that the larvae can indeed hold temperature below 16°C for days, Authors also describe the possible reduction of food intake by larvae with long exposition to more than 35°C, leading to increased physical inactivity, followed by death (Chia *et al.*, 2018). Nevertheless, those references are associated with a specific BSF strain and the strain used by ENTOMO has been probed to hold temperatures of 36°C continuously for a period of 10 days without showing a deleterious effect on the population.

Despite affecting mortality increase, breeding insects (BSF in particular) under unfavourable conditions is possible, but there is a significant loss of efficiency. All stages of the life cycle are impacted and consequently it takes more days to finish the cycle.

For example, the development time required for a newly hatched neonate larva to become an adult fly is about 28 days when subjected to the whole process at 30°C. However, this value increases to 184 days if the same cycle is managed at 15°C (Chia *et al.*, 2018).

The Black Soldier Fly occurs naturally in a very wide range worldwide, in all regions between the latitudes of 40°S and 45°N (Dortmans, 2017), which in Europe, including most of the Mediterranean area. However, the manufacturing rearing of BSF in higher latitudes is, so far, dependent on a controlled environment, to keep under control the temperature, relative humidity, and the parts per million of carbon dioxide present in the air, and therefore reaching successfully the ideal full-cycle propagation.

During the larvae stage, up to 7 instar changes happen. After reaching a certain size – the time for it to happen will vary according to density, diet, etc, - the larvae with approx. 8±3 mg (stadia 3 or 4, Figure 5, show a decrease in the metabolic rate and start to concentrate efforts on building body mass and storing the energy reserves (anabolism) that will be necessary for metamorphosis into an adult fly and, therefore, for reproduction (Gligorescu *et al.*, 2019).

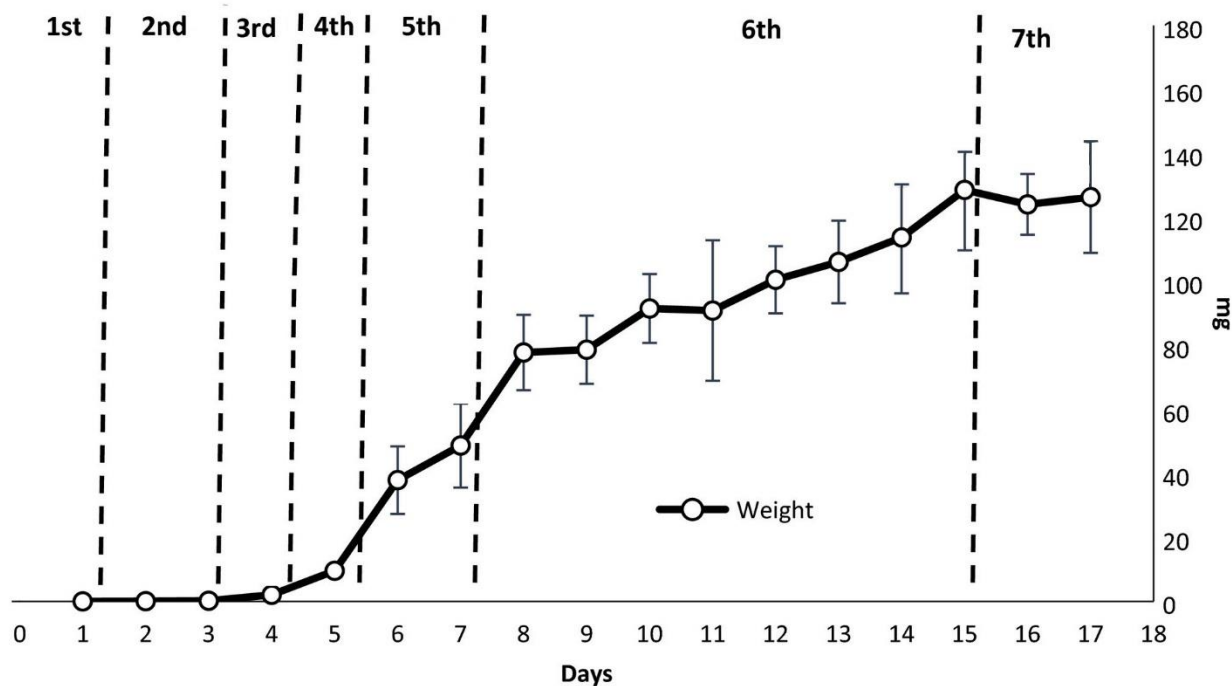


Figure 5. Growth in mass (mg) of *Hermetia illucens* larvae measured daily (mean  $\pm$  SD). Larval stadia 1–7 is delimited by vertical dotted lines based on statistical analysis of head capsule widths. (Adapted from Gligorescu *et al.*, 2019)

In a summary, it can be concluded that *Hermetia illucens*, Figure 6; **Error! No se encuentra el origen de la referencia.**







-  ➤ It is an excellent organic matter converter
-  ➤ It is a cosmopolitan species widely distributed throughout the planet, and registered in Spain in 1954 and in Europe in 1500's.
-  ➤ Its reproductive capacity and adaptation to biotic and abiotic conditions, allow it to modulate its development and rapidly multiply its population.
-  ➤ Larvae can feed on a wide variety of organic matter, with different dry matter content from 2% to 30%, being able to eat solids, sludges and liquids. Very useful for the treatment of vegetables and microorganisms.
-  ➤ It can reduce the volume of waste in high proportion, easily achieving 60% reduction in weigh bases
-  ➤ The larvae prevent the proliferation of microorganisms such as *Escherichia coli*, *Salmonella spp.* among others. It is very useful as sanitation of waste and to prevent the spread of diseases

Figure 6. BSF Attributes

### 3 TECHNICAL REQUIREMENTS AND ACTIVITIES

In this section, ENTOMO describes the requirements that have to be fulfilled in order to be able to close the production cycle of the species in an efficient way. Since the IPP are based on BSF larvae production, this document develops about this species requirement. However, the same conditions are desired for any other insect species and with little modifications the same production space could be converted into a new insect species production. After the bibliography study of the specie biology and understanding the life cycle, ENTOMO has carried out experiments to better know the duration of every stage at different temperatures, humidity and densities. To do that, ENTOMO have been using local strains of wild *H. illucens* occurring naturally in organic waste piles (manure) collected in Murcia. With the larvae collected, a laboratory population was created and domesticated for production and studies.

This section starts with the premises, reflecting the summary of the results after studying the used strain and dividing the insect cycle in the different phases for insect farming. After that, an explanation of the activities done in insect production (breeding, hatchery and nursery, fattening, processing) is described. Finally, an explanation of the risks is included to be considered in the setting of the IPP.

#### 3.1 Premises

It has been decided to use this strain because have been found locally and thus it is expected to have adaptative conditions to Murcia and Mediterranean conditions. The population used by ENTOMO have been studied and the following parameters describe the strain characteristics.

1<sup>o</sup>) Optimum temperature: 5 different temperatures were tested (all the temperatures at a fixed relative humidity (RH) of 85% in each stage (Adults, eggs-hatchlings, larva and pupa) and the time needed in each stage and survivability rate was recorded. From that experiment it was concluded that optimum temperature is 29°C for eggs-hatchlings (time 2.5+5 days), 28°C for larva raising (12 days), 28°C for prepupa (12 days), and 29°C for adults (10 days).

2<sup>o</sup>) Optimum RH: In a similar experimental setting to the former experiment, every stage was expose to 4 different RH, 60%, 70%, 80%, 90% and with the temperature corresponding to the optimum calculated before. Survival and normal animal performance were registered. The results showed that eggs-hatchlings cannot survive with RH below 80%. Adults did not release eggs at a lower RH of 65 %, Larva was not affected by the RH nor pupa and pre pupa.

3<sup>o</sup>) Animal density: since every stage needs a different cage/recipient and density could really influence animal performance optimization of density was determined by putting different densities in a standard container. At the end of the cycle (time obtained in experiment 1). Only adults, egg-hatchling and larva were considered since pupa and prepupa don't move so much and do not interfere with each other. As a result, maximum density of larva was about 12 kg/m<sup>3</sup> and adults 600 individuals per litre. Hatchlings were not affected by density although at too high density the development is delayed resuming normality when the density is readjusted.

4<sup>o</sup>) Capacity to eat from liquids: to test the capacity of the larva to be feeding in diets solid, semisolid or liquids, an experiment was carried out by preparing a diet of brewer yeast, wheat meal and corn meal at 3:4:4 and 5 different dry matter content 2%, 5%, 10%, 20% and 30%. With a supporting structure and without the support structure. Larva was able to feed at all water content diet as far as they had the support structure. Dry matter content of 2%, 5% and 10% was impractical without the support structure. 30% dry content diet delayed larva development and optimum was achieved at 20% dry matter content. Diet volume was reduced from 50% to 65% along the time.

The production activities are based in treating each life cycle in specific conditions to achieve maximum production efficiency, it is shown in Figure 7

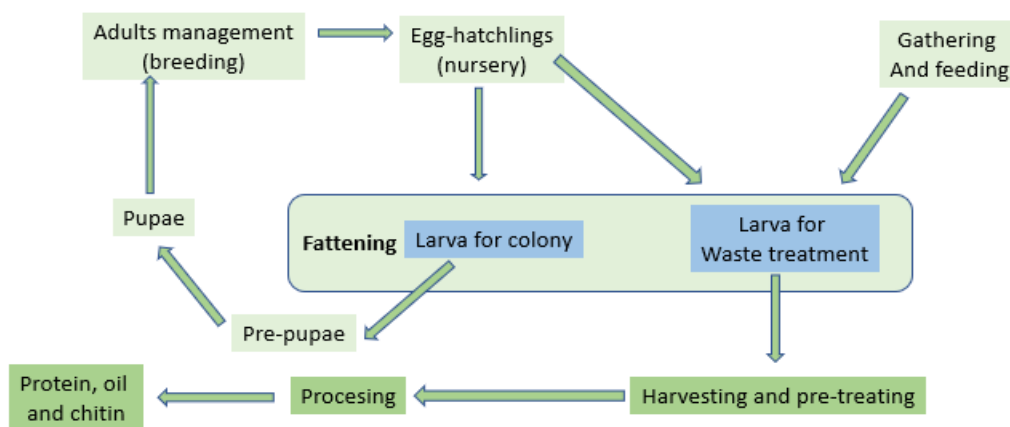


Figure 7. Production cycle and activities division

### 3.2 Breeding

The development of adults will take place in cages specially designed for this stage of the insect. The cages will be independent, made of insulating material, with a glazed area to facilitate the entry of natural light and favour the copulation and oviposition of the adults. Inside, the following elements will be arranged:

- Package with 30,000 pupae
- Container with an attractant (based on vegetable by-products) with a mesh to favour the exposure of the smell of organic matter
- Oviposition substrate
- Container with water for adults

Daily, the eggs are removed from the adult cages (see pictures of the adult cages in the results) and will be carried out by accessing various areas of the cage from the outside. This will avoid disturbing adults and possible contamination of personnel accessing the premises. The potential escape of adults is a critical point in the production program of the colony. However, as already explained, this species does not imply a pest for humans and animals, or a vector of parasites or diseases. Despite this, prevention and action

measures have been established in the event of an adult escape (see Annex I). The adult room will have a controlled temperature of 30 °C, and an area of 20 m<sup>2</sup>.

### 3.3 Nursery

Once the eggs are collected, they are transferred to the Hatchery Hall, 10 m<sup>2</sup>. There they are arranged in groups on various containers with food in the Incubation Rooms. These rooms are equipped with temperature, humidity, CO<sub>2</sub> and lighting control, to establish the ideal conditions for larval emergence, about 28 °C and 75% RH. A specific diet is prepared for the hatchlings (a defined composition of cereals and vitamins) and arranged in several containers. The eggs are placed just over the fresh food. The smell that gives off the food and the conditions of the room facilitates the emergence of the larvae. After three days of incubation, the larvae emerge, fall on the food and stay 5 days in this room. To prevent larval escape, it is very important to establish a vigilance plan, observe the shelves, packaging damage, etc. When the larvae have 5 days of development (stage L3), the larvae are divided in two, 5% will be used for breeding population (feeding on nutritious diet) and 95% will be sent to eat by-products in the fattening area.

### 3.4 Fattening

The 5% of the larva to keep the breeders, are introduced in the colony room where they will stay until they reach pre-pupae stage. This is the stage that consumes more food, and is more important for the accumulation of fats and proteins in the larva which is required to generate a robust and fertile adult. The other 95% will be fed with the by-product diet until harvest time. In both cases, the fattening rooms are equipped with temperature, humidity, CO<sub>2</sub> and lighting control, to establish the ideal conditions for the development of larvae, about 28 °C and 65% RH. Under these conditions, the larvae will be in the room for 12 days.

### 3.5 Gathering and feeding

The organic matter (substrate) that is used to feed the larvae, will be stored in the digestate reception deposit. From there either it will be used right straight to feed the larvae or will be mixed with other components. The preparation of the food is necessary for the maintenance of the population and efficient recovery of by-products.

Upon receipt and handling of by-products, the area and machinery used will be disinfected. The experimentation room will have an area of 21.12 m<sup>2</sup>.

### 3.6 Pre-pupa development

After 12 days in the fattening rooms, the larvae are moved to a special structure to promote to pre-pupae. In this structure, the larvae are fed for 2 more days. The larvae are then chitinized, darkened, and the prepupa is fully formed. They are then collected and mixed with peat at 50% of the volume, to provide shelter for pre-pupae to continue their development.

### 3.7 Pupa development

After 7 days in the pre-pupa development room, the pupae move to the Adult Room. In this room they are kept in controlled conditions for another 7 days. After this period, they move to a cage where adults will emerge, and the cycle will be repeated. This room is equipped with temperature, humidity, CO<sub>2</sub> and lighting control, to establish the ideal conditions for the optimal development of the pupa, about 30 °C and 65% RH.

### 3.8 Quality control

The facilities have a laboratory with entomological material and equipment to carry out quality control. Among the tasks to be carried out, the following stand out:

- Tasks for quality control of biological material
- Inspection of lots, colour traps, baits, and other pest monitoring devices
- Inspection of pests and parasites detected
- Disease diagnosis
- Freezing of discarded batches
- Testing for composition levels

### 3.9 Machinery

Each process needs the use of certain machinery to ease the work and to allow stable conditions. In Table 1. Machinery and equipment used in the production phases Table 1. It is described the type of machinery used in each stage of production.

Table 1. Machinery and equipment used in the production phases

Phase	Element/Activity	Machinery	Infrastructure
<b>Feed gathering and feeding</b>	Download and handling of the food storing	- Grinder - Mixer - Hammer mill	- Concrete floors - Deposits and containers, valves and pipes
<b>Adults</b>	Cages management and maintenance	- water supply system - Air compressor	- 33 m <sup>3</sup> capacity cages with insulated walls - Temperature and humidity-controlled rooms, specific artificial lighting
<b>Egg's incubation</b>	Batch preparation	- food delivery system	- Climatic rooms, with controlled T <sup>a</sup> , RH, CO <sub>2</sub> and light - Shelves for incubating boxes
<b>Larva Nursery</b> <b>Larvae fattening</b>	Batch movement	- Feed delivery system - Trolley for transport - Trolley for larvae transporting - Automatic sieving - Scales	- Climatic rooms, with controlled T <sup>a</sup> , RH, CO <sub>2</sub> and light - Climatic rooms, with controlled T <sup>a</sup> , RH, CO <sub>2</sub> and light - Shelves for incubating boxes
<b>Pupae development Laboratory</b>	Quality control activities	- Precision scale	- Climatized room

Phase	Element/Activity	Machinery	Infrastructure
		<ul style="list-style-type: none"> <li>- Microscope</li> <li>- Spectrophotometer</li> <li>- Incubator</li> <li>- Freezers</li> <li>- Dryer stove</li> <li>- Oil extruder</li> </ul>	
<b>Disinfection tasks</b>	Area cleaning and disinfection	<ul style="list-style-type: none"> <li>- Pressurized water system</li> <li>- Steam pressure cleaner</li> <li>- Industrial aspirator</li> </ul>	<ul style="list-style-type: none"> <li>- All the areas</li> <li>External areas for cleaning large recipients</li> </ul>

### 3.10 Risk Management

When working with live animals and more specifically insects many incidences might happen to affect the production at several levels:

- Workers (hazards)
- Produced insects (infections or infestations)
- Environment (massive insect escapes)

The hazards that could affect the workers are mainly related to allergies, and could be prevented with respiratory protection system as masks with adequate filters. Other protection such as globes and adequate clothing can prevent small accidents that could create discomfort.

The risks related to insects are described in Table 2.



Table 2. Risk description and its management in insect operations.

Phase	Element / Activity	Risk	Preventive Measures	Corrective Measures
<b>General considerations</b>	State of the enclosures	- Entry of pests - Escape of individuals	- Staff training: Review - Contracting a maintenance service	- Proceed to immediate repair - Follow the indications of other associated risks
	Power interruption	- HVAC system shutdown - Stoppage of the feeding system	- Installation of an electric generator, and independent basic supply lines (ventilation, food transfer). - 24-hour service contract	- Contact with the energy supplier - Service notification
	Serious illness of personnel	- Risk of disease spread	- Flexible shift design - Regular medical check-ups	- Isolation of affected personnel - Monitoring of health guidelines - Temporary staff support
<b>Collection and treatment of by-products</b>	Unloading and handling of feed	- Tipping on sill	- Staff training	- Collection, disinfection and dewatering to sewerage system
		- Contamination with other insects	- Handling under controlled conditions	- Stockpiling in anaerobic conditions of the batch
	Feed storage	- Rodent and bird infestation	- Trap placement and monitoring - Widespread cleaning and disinfection methods	- Checking of tanks and storage tanks - Sanitisation measures - Reinforcement of the plan
		- Contamination with other insects	- Handling under controlled conditions - Trap placement	- Storage in anaerobic conditions of the batch - Assess discarding of the batch - Temporary rental of machinery
	Rinsing and treatment of feed	- Breakdown of the transfer machinery - Breakdown of crushing equipment - Breakdown of mixing equipment - Electrical breakdown in the installation	- Staff training - Proper maintenance of equipment - 24-hour repair service	
	Lack of feed	- Inability to feed batches	- Prepare food stockpile	- Apply lethargy to neonate larvae - Follow contingency plan
<b>Adults</b>	Disposition of attractant	- Pest contamination of the attractant	- Preventive measures during collection	- Recipient freezing - Cage disinfection
	Removal of eggs	- Escape of adults	- Monitoring of dipteran traps - Checking of cages - Handling at times when adults are dormant	- Location of the exhaust. Closing the opening - Irrigation and aspiration of adults - Completion and disinfection of



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Phase	Element / Activity	Risk	Preventive Measures	Corrective Measures
Hatchery	Weighing and disposition of eggs	- Dropping or forgetting batches during activity	- Staff training - Review of the work area at the end of the task	the affected cage. - Collection of eggs and/or hatching larvae - Disinfection of the area if hatching larvae have emerged
	Feed disposition and evolution	- Contamination with other insects - Contamination with fungi and bacteria	- Handling under aseptic conditions - Review of the evolution of the food	- Disposal by freezing of the batch concerned
	Larval development	- Escape of larvae out of containers	- Checking of packaging - Surveillance of the area	- Collection of larvae and disinfection of the room
		- Falling containers	- Correct placement of containers - Training: avoiding knocks and sudden movements	
		- Toxin-related mortality	- Review of food development	- Disposal of the affected batch
Hatchery (cont.)	Larval development (cont.)	- Presence of a disease	- Hygiene measures - Positive pressure in rooms - Separation of areas, activities and batches	- Identification of the disease - Disposal of the infected batch - Disinfection of the room - Application of measures according to pathogen
Hatchling development	Weighing and disposal of larvae	- Escape of larvae during the activity	- Staff training - Review of the work area at the end of the task	- Larvae gathering - Area disinfection
Larval fattening  Pre-pupae development	Food provision and development	- Contamination with other insects  - Contamination with fungi and bacteria	- Handling under aseptic conditions - Review of the evolution of the feed - Monthly disinfection of the rearing chamber	- Disposal by freezing of the batch concerned
	Evolution and transfer of larvae	- Escape of larvae out of containers	- Checking of packaging - Surveillance of the area - Searching for nooks and crannies in the room	- Collection of larvae and disinfection of the room or of the transport
		- Falling containers	- Correct positioning of containers - Avoid bumps and knocks	- Collection of larvae and disinfection of the room or of the transport
		- Appearance of larval parasites	- Fitting of screens and filters in ventilation systems - Positive pressure in rearing and	- Identification of entry points - Disposal of affected batches - Disinfection of room or transport - Subsequent tracing of matched



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Phase	Element / Activity	Risk	Preventive Measures	Corrective Measures
			transport chambers - Daily monitoring of traps	batches in the room or in transport
		- Toxic mortality (chemical or biological)	- Review of the evolution of the food - Cooking of the food	- Freeze disposal of the affected batch- Identification of the poison
		- Presence of a disease	- Hygiene measures - Positive pressure in rooms - Separation of areas, activities and batches	- Identification of the disease - Disposal of the infected batch - Disinfection of room or transport - Measures according to pathogen
<b>Pupae development</b>	Pupae development	- Escape of pre-pupae from pupation containers	- Checking of packaging  - Surveillance of the area  - Searching for nooks and crannies in the room	- Collection of pre-pupae and disinfection of room or transport
		- Leakage of adults from packaging	- Monitoring of dipteran traps in the room - Checking containers for escape hole - Handling at times when adults are inactive	- Location of the exhaust. Closing the opening - Irrigation around the leakage area - Vacuuming of adults - Disinfection of room or transport
<b>Quality Control</b>	Sieving procedures	- Ineffective phase separation	- Adequate monitoring of the batch - Equipment maintenance	- Repeat refining - Addition of sieves - Batch re-feeding
	Monitoring of indicators	- Deviation from expected values	- Implementation of procedures - Prior monitoring of the indicators for each stage of the process	- Activation of traceability and correction procedures - Implementation of necessary corrections
<b>Product generation</b>	Product transfer	- Tipping on sill	- Staff training	- gathering and destruction according to protocol
		- Detection of contamination	- Handling under controlled conditions - Staff training	- Batches destruction - Detection and destruction procedure application
	Stopping the production cycle	- Material jammed in machinery	- Strict maintenance plan - Staff training	- Assessment of the ability to continue production - Shutdown, cleaning, disposal of the product - Reuse of material where possible
		- Material to be processed		- Conservation according to



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Phase	Element / Activity	Risk	Preventive Measures	Corrective Measures
				procedure to prevent deterioration of the material
	Review of control parameters	- Efficiency reduction or cross-contamination	- Strict maintenance plan - Staff training - Pre-screening of incoming material	- External analysis of products - System shutdown, and review - Review biological traceability
	Storage of products	- Occurrence of pests	- Placement and monitoring of traps  - Widespread cleaning and disinfection methods	- Review of storage tanks and reservoirs - Sanitation measures - Reinforcement of the plan
		- Expiry of batches	- Exhaustive numbering, registration and tracing of lots - Sale output	- Prioritising the sale of old batches - Analytical controls to monitor quality parameters



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## 4 LOCATIONS CHARACTERISTICS

As part of this project's objectives and to promote circular economy, small insect production facilities in the Mediterranean area seem to make economic sense. This chapter it is first presented a general consideration of weather and small-scale production in the Mediterranean countries, followed by summarised conclusion obtained after the observation of the information collected from each location for the IPP (Turkey, Tunisia, Italy and Spain).

### 4.1 Generalities in weather and insect production

Small facilities for insect rearing and processing at the scale from some kilograms to a few tons per day, is very feasible for a small independent business located close to the waste source and the poultry farm. Insect production has the great advantage of not needing a large space for breeding, thus no need for large investment, but the main problem is the need to supply good abiotic factors such as temperature, relative humidity, carbon dioxide ppm, etc. in order to achieve better yields. It makes it very unfavourable to set up insect production farms in locations where temperatures drop below 15°C for a too prolonged period of time and only by the installation of Humidity Ventilation and Air Conditioning (HVAC). Thus, the location where it is desired to start breeding does not have the natural conditions, only by means of HVAC installations will it be possible to carry out such a business.

In places with partially favourable natural conditions, such as some areas of the Mediterranean (see Figure 8. Graphic showing the temperature conditions (maximum, average and minimum) in Murcia city, along the Mediterranean basin in Spain year of 2019. Data extracted from CARM.es(Figure 8), temperatures are within the range required for the production of BSF (between 25°C-37.5°C) for a relatively long period of the year (from May to October), and the portion of the year with temperatures below the requirements the temperatures very seldom goes below 5, thus the requirement for heat is not excessive. The main problem in the summer months is the extremely low humidity often below 40% RH and much lower than needed for adult's egg production (65-80%), however, humidity is less relevant for the larval stage since the moisture content of the diet (70%) can be easily controlled.

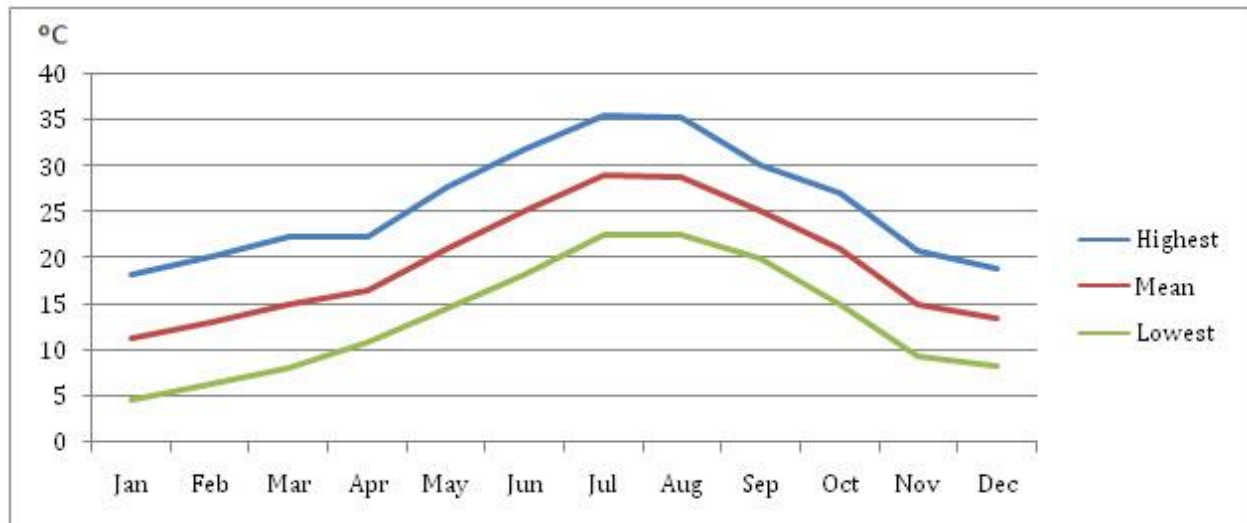


Figure 8. Graphic showing the temperature conditions (maximum, average and minimum) in Murcia city, along the Mediterranean basin in Spain year of 2019. Data extracted from CARM.es

The BSF life cycle stages that require the most care, and whose non-provision of adequate conditions can severely affect survival and proliferation, are:

- the adult stage, and consequently the mating moment and oviposition, registering more than 40% fewer eggs deposited if the temperature is 20°C and not 30°C
- the hatching of eggs – for instance, failing to hatch at 12°C or with humidity below 50%;
- and the neonate larvae (hatchlings) during its first 5 to 7 days.

Considering the supporting data, a small insect rearing farm can be carried out in locations with favourable environmental conditions, where the minimum investment should be concentrated on the most vulnerable stages of the cycle (adult, egg and hatchlings).

Production experience with Entomo AgroIndustrial's laboratory has shown that at this stage, moult between the 3rd and the 4th larval stadia, called internally "seeding larvae", the fattening and exposure to different types of diets, as well as changes in abiotic parameters, such as temperature, relative humidity and carbon dioxide concentration, are better tolerated by the larvae than in early instars.

The occurrence of BSF naturally in the Mediterranean, associated with a possible increase in its occurrence due to climate change scenarios, opens an important window of opportunity to associate the rearing and fattening of soldier fly larvae with traditional livestock husbandry practices. The possibility of expanding the production of insect protein in open environments, on a small or medium scale, associated with the management of organic waste, would meet the expectations of strategies to support the Circular Economy, while enabling small rural producers to obtain self-sufficiency in animal feed, an important aspect associated with Sustainable Development Goal 10: Reduced Inequalities.

## 4.2 Turkey

The purpose of analysing the potential in each location is to plan the capacity of the IPP to produce the larvae in order to get the maximum capacity, enough to carry out the poultry feeding trials. Climate conditions help to decide the capacity of the HVAC system and therefore the needs of electricity depending on the needs of energy.

As the first factor to consider is the number of by-products available all through the year. In the case of Turkey, it can be appreciated that from January to June the total sum is 792 kg/day of by-products; July to September total sum is 1792kg/day; and from October to December total sum is 10792 kg/day. The main sources are vegetables or soup with little dry matter and only bread could be considered a concentrated source of nutrients. Because of this low concentration, we would consider a conversion of 1/16, so one kg of larvae per every 16 kg of by-products. Another consideration is dry matter of larvae at harvest time to be 33%. Thus, the expected production of dry larvae will be 16,5 kg/day from January to June; 37kg/day from July to September; and 225kg/day from October to December. As can be observed, even at the lowest production, it is possible to achieve a total of 500kg/month of dry larvae to feed the poultry which could be achieved with a container size pilot.

In terms of climate although no parameters are perfect for the production at the same time. The conditions are soft enough for considering insect production.

- Temperatures: Only favourable from the months from May to October. However, temperatures are not extremely low. It would be possible to produce the insect with the help of greenhouse to increase the temperature during the coldest months. Another measure would be to reduce production in December and January. Adults will need extra heat in order to continue ovipositing.
- Humidity: good humidity balance, just being low during hot months. Thus, humidifiers have to be incorporated in adults and hatchery rooms. Larvae will not be affected.
- Light will have to be complemented during the months with light below 12 hours/day.
- 

In terms of water and electricity supply is adequate for the scale of the pilot

## 4.3 Tunisia

Considering diet as the main factor for the IPP. In the case of ISA-CM, it is going to use the canteen waste which is available year-round with exception of holidays. Thus, approximately 34Kg/day of waste is achieved with exception of the months of April, July, August and December. The type of foodstuffs like bread, pasta, legumes and fruits are quite condensed in nutrients and then it would be expected to have a conversion of 1/7, so 1 kg of larvae per every 7 kg of canteen waste. Giving a production of larvae of 4,85kg of larvae/day. Which in the dry form will give 1,62kg/day. The production is expected to be 48 kg/month. The container IPP will achieve this production easily. For the case of Rayhana, production is not relevant and the IPP will be dedicated to dissemination of the technics to small farmers, household and small enterprises. However, results will be track to corroborate the success of the implementation.

- Temperatures: Only favourable from the months from June to September. Lower temperatures are always above 7, allowing for a year-round production if it is assisted with passive solar heating
- Humidity: showing a very sharp peak, with special attention in lower peaks that will have to be corrected with humidity control systems.
- Light will have to be complemented during the months with light below 12 hours/day.

In terms of water, it seems to be scarce and with a high load of salts, only good for cleaning, sanitizing and air conditioning. But for diet preparation will need to be depurated by osmosis. However, due to material scarcity, the suggested water cycle will be, inlet water to be pass by osmosis, the purified water to be stored and the rejection (loaded with more salts as well. The rejected water will be used for cleaning

In terms of electricity, for the limitations in the supply, the container will be provided with solar power to supplement as much power as possible and aim to supply the 100% of the needs. The design will be also focused in low energy consumption, sacrificing production to make the pilot more sustainable. However, a connection to the general supply will be used as per emergency or contingency source.

#### 4.4 Italy

In the case of Torino (Italy) PPI situation will be analysed as a complementary for the SUSTAvianFEED project. According to the information provided by UNITO, there is a fairly good number of organic by-products in the area which could successfully be used in the production of BSF. By volume, fruit pulp, carrots and potato peels are the most abundant and fairly complemented with eggs, whey, yeast and beer bagasse. All products give approximately 20 tons/day of by-products. Considering the potential as a feed source of each ingredient used it can be anticipated a total production of about 3.5 tonnes of live larvae per day, fully grown with a dry matter content of 35%. Thus, giving a production of 1 tonne of dry larvae a day.

- Temperatures: are relatively cold for the needs of the larva and only appropriated during daytime on summer days. Many days the temperatures are below 0. Fortunately, no cooling system will be needed because temperatures never exceeded the biological maximum only needing the investment in heating, which is cheaper than air conditioning.
- Humidity: Adequate all through the year, and easy to supplement with simple systems.
- Since the temperatures are low and production in chambers will be necessary, artificial lights will be needed.

No more parameters have to be analysed in this case



## 4.5 Spain

For the IPP of Spain, it has been selected as the main food source beer bagasse and being complemented with vegetable trims and milk whey. In total will be 22 tons a day of organic by-products with almost 50/50 beer bagasse/vegetables and supplemented with the milk whey proteins. It could be expected to obtain a production ratio 1/6, so 6 kg of feed to obtain 1 kg of larvae, which will achieve an 85% size with a dry matter content at harvest of 30%. It gives a total production potential of 1100 kg of dry larvae.

- Temperatures: Only favourable from the months of June to September. And being necessary to supplement heat during the nights and in the winter months. It can also be observed very high temperatures in the hottest months. For this reason, it is necessary the installation of air conditioning. Being with good energy supply, a HVAC with cooling and heating is the best option to satisfy summer and winter needs. Enthalpic recuperators help with energy saving by recovering the enthalpic energy of the air during air renovation.
- Humidity: Can be considered low and it would interfere with many physiological activities like eggs oviposition or larvae development. Thus, steady humidifiers have to be installed to assure the right humidity levels.
- The light is similar to other locations. However, since the production have to be done indoor due to inadequate temperatures, artificial lighting will be used.
- Water quality is good, neutral with a relatively low level of salts. Supply is secured and it is not a concern
- The facility is in an industrial area and with a contracted supply of 50Kw, triphasic and monophasic so the supply is guaranteed for the machinery to be working at the decided production scale.

## 5 GENERAL DESIGN PROPOSITION AND ACTION PLAN

With the data collected from the partners the decision agreed by the partners is to create 3 physical IPP, Tunisia for medium-scale (container in ISA-CM) and small scale (domestic pilot), Murcia for the intensive scale.

### 5.1 Big scale (Spain)

For this IPP the Pilot already existing in Murcia will be used. It will be dedicated partially to this project and has the following characteristics shown in Table 3. It is installed in Cehegin (Murcia) in a warehouse in an industrial area. The production capacity will be 50 kg of dry larva per day. In the case to achieve this production when the larvae are fed a diet of beer bagasse, and vegetables.

Table 3. Space distribution used in each production area

Area	Surface (m <sup>2</sup> )	Description (location)	Characteristics	Equipment conditions
<b>Adults room</b>	20	Adults' area for mating and female oviposition, eggs harvesting is facilitated (Entomo Research Centre)	Climatized rooms to keep the temperature in the range 28 to 30°C, programable air renovation, RH at 75%. Artificial light with specific spectrum	- Cages 33 m3 - T <sup>a</sup> , HR and Light photoperiod controlled
<b>Experimental room</b>	20	Room to carry out the organic urban waste tests. (Entomo Research Centre)	Clean and climatized at 30°C and 65% RH	- Air renovation equipment dehumidifier and temperature control Structural support for larva fattening and testing boxes
<b>Production room</b>	25	Room to carry out the organic urban waste treatment (Entomo Research Centre)	Clean and climatized at 30°C and 65% RH	- Air renovation equipment dehumidifier and temperature control Structural support for larva fattening and testing boxes
<b>Colony room</b>	25	Room to fatten larvae to keep the colony and to produce products (Entomo Research Centre)	Clean room climatized at 30°C and 65% RH	- Air renovation equipment dehumidifier and temperature control Structural support for larva fattening and testing boxes

Area	Surface (m <sup>2</sup> )	Description (location)	Characteristics	Equipment conditions
Laboratory	40	Laboratory for QC activities, testing, samples analysing, washing and biological material manipulation (Entomo Research Centre)	Air-conditioned rooms. Easy clean no slippery floors	<ul style="list-style-type: none"> <li>- Sieves</li> <li>- Fridge for samples</li> <li>- Precision Scales</li> <li>- Lab furniture</li> <li>- Material</li> <li>- Agitator, blender</li> <li>- Aire conditioner</li> <li>-500 litres oven/dryer</li> <li>- Oil Extruder</li> </ul>
Hatchery	14	Room for hatchling and nursery from egg to day 5 old larvae (Entomo Research Centre)	Clean room climate room at 30 °C and 75% RH 2.88 x 4.96	<ul style="list-style-type: none"> <li>- Air renovation equipment</li> <li>dehumidifier and temperature control</li> <li>Structural support for larva and trolley</li> </ul>

The ENTOMO's IPP is shown in the Figure 9

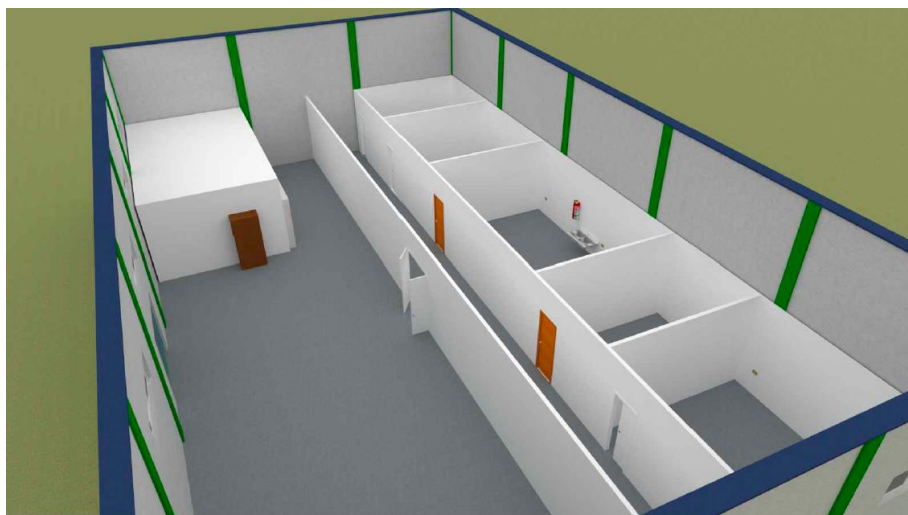




Figure 9. The ENTOMO's IPP to be used during the project execution. Up, General view in 3D of the IPP; down left internal corridor connecting with the chambers and; downright the production structure

## 5.2 Medium Pilot scale (Tunisia)

For this IPP in Tunisia to be installed in ISA-CM it will be use a container model serving as an entomological laboratory and it will include all the infrastructure to achieve processing of 34kg of waste a day and to generate 1.6 kg of dry larvae in the same period. It will have a small laboratory to do larvae feeding trials and some other QC measurements. The container will have a reverse osmosis system, water storage for fresh and clean, and solar panels to power the container. See Table 4

Table 4. Space distribution used in each production area Tunisia IPP

Area	Surface (m <sup>2</sup> )	Description (location)	Characteristics	Equipment conditions
Adults room	2	Adults' area for mating and female oviposition, eggs harvesting is facilitated (ISA-CM)	Climatized rooms to keep the temperature in the range 28 to 30 °C, programable air renovation, RH at 75%. Artificial light with specific spectrum	- Cages 1 m3 - T <sup>a</sup> , HR and Light photoperiod controlled
Production room	14	Room to carry out the canteen waste treatment and feedstock/harvesting processing (ISA-CM)	Clean and climatized at 30°C and 65% RH	- Air renovation equipment dehumidifier and temperature control

Area	Surface (m <sup>2</sup> )	Description (location)	Characteristics	Equipment conditions
				Structural support for larva fattening and testing boxes
<b>Colony room</b>	2	Room to fatten larvae to keep the colony and to produce products (ISA-CM)	Clean room climatized at 30°C and 65% RH	- Air renovation equipment dehumidifier and temperature control Structural support for larva fattening and testing boxes
<b>Laboratory</b>	8	Laboratory for QC activities, testing, samples analysing, washing and biological material manipulation (ISA-CM)	Air-conditioned rooms. Easy clean no slippery floors	- Sieves - Fridge for samples - Precision Scales - Lab furniture - Material - Agitator, blender - Air conditioner/dryer - Oil Extruder
<b>Hatchery</b>	4	Room for hatchling and nursery from egg to day 5 old larvae (ISA-CM)	Clean room climate room at 30°C and 75% RH  2.5 x 1.6	- Air renovation equipment dehumidifier and temperature control Structural support for larva and trolley

An illustration of what will be the ISA-CM's IPP is shown in the Figure 10, Figure 11 and Figure 12. The images are showing a previous constructed container where it can be seen an external façade with windows to allow showing the works inside and an iconography view which could show the SUSTAvianFEED approach. Similar model will be created for ISA-CM.



Figure 10. Typology of IPP to be constructed and installed in ISA-CM: Up-left, external view of the container where the walls act as illustration for the project iconography; Down-left, interior part of the container showing fattening area and; Right, breeding ca



Figure 11. Inside view of the container: Left area for feed preparation and larvae extracting and cleaning; Right, laboratory for QC activities and assessment.



Figure 12. Inside view of the container: Left area for feed preparation and larvae extracting and cleaning; Right, laboratory for QC activities and assessment.

### 5.3 Small farm/household scale (Tunisia)

This IPP pilot has been thought to be installed in Rayhana, and it will serve more as a demonstration site for technology transfer, rather than a productive site. ENTOMO will prepare material based on locally available commodities in order to be easily replicable and scalable for at household and small farmer scale. It is expected to serve as a seed food for thoughts in order to encourage the insect farming activity in the area where Rayhana acts or even spread to other locations.

Details of the material used and handbooks for distribution will be provided and added to the revised version of this deliverable after the first visit to Tunisia.

## 5.4 Planning

General planning of this task implementation is described starting with Figure 13.

Insect Pilot Planning			2021												2022											
			abr-21	may-21	jun-21	jul-21	ago-21	sep-21	oct-21	nov-21	dic-21	ene-22	feb-22	mar-22	abr-22	may-22	jun-22	jul-22	ago-22	sep-22	oct-22	nov-22	dic-22			
SUSTAvianFEED Gantt Chart		Lead Partner	Year 1												Year 2											
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21			
1	Insect Pilot construction	ENTOMO																								
2	Pilot transportation and installation	ENTOMO																					D3.1			
3	Larvae production for the pilots	ENTOMO																								

Figure 13. Gantt for IPP activity

### 5.4.1. Insect PILOT construction (M10-M15)

In ENTOMO's facility the pilot in a container form will be constructed and tested to ensure everything is working properly considering the following criteria

- **Partner needs** of dry insects to carry the feeding the poultry feeding pilot.
- **Remote control** to allow the operation and troubleshooting from Spain
- **Low consumption** of water and energy to have a low impact on partners expenses

The pilot will start to be constructed in M12 and tested from M12 to M14

### 5.4.2. Pilot transport and installation (M15-M20)

It is not yet clear when the pilot will be finally shipped, but it is estimated to be in mid-August 2022 and 1 month transport is anticipated.

In mid-September the pilot will be started up and in parallel training will be provided by Entomo's team to:

- **ISA-CM:** to personnel and student involve in the insect rearing
- **RAYHANA:** Associates or trainers who will train the women farmers to operate their insect operation

The summary of the three IPP is shown in Figure 14



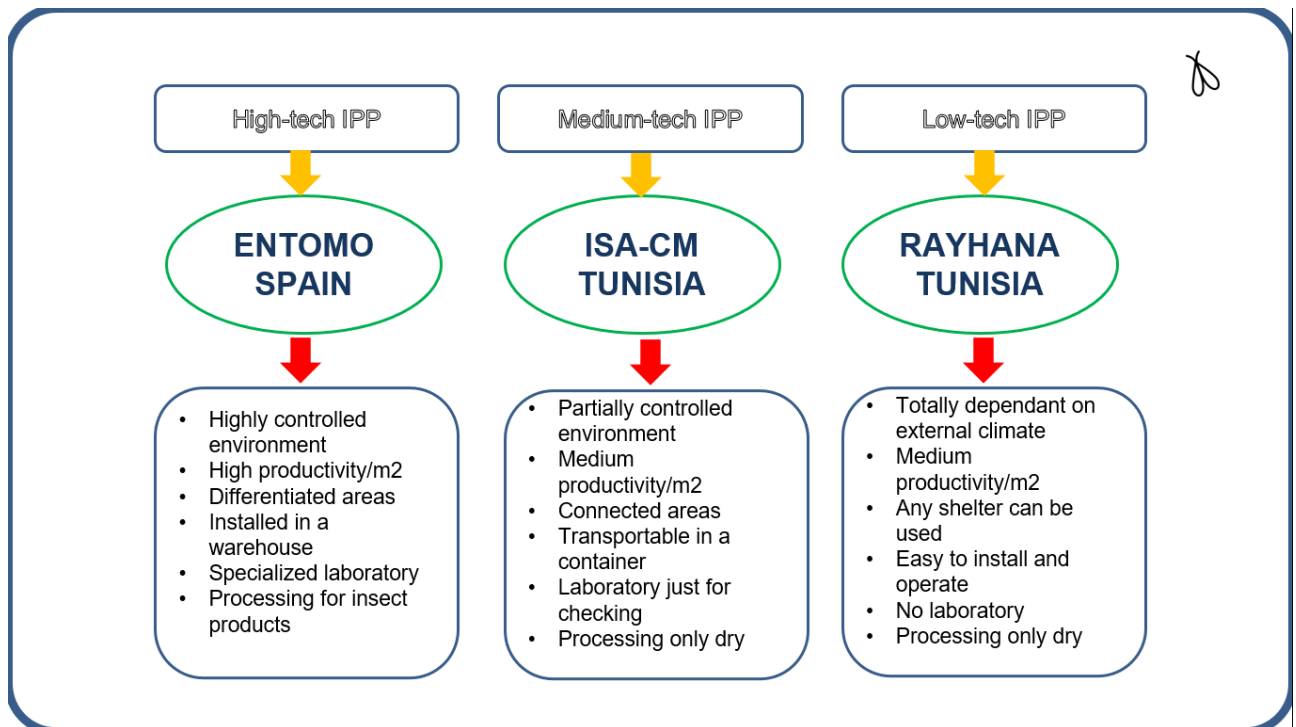


Figure 14. Summary of IPP characteristics

### 5.4.3. Larvae production for the pilots (M10-M20)

In parallel to the activities of insect pilot construction and installation ENTOMO will produce and provide dry insects to the partners who need them to carry out the poultry feeding trials and the experiments.

#### Plan by items in table 5

Table 5 shows the planification for the IPP

Table 5. Planning of the IPP

No. Item	Actions to be conducted	Responsible partner	Deadline
1	Insect PILOT construction	ENTOMO	28/06/2022
2	Planning of dry insects needed for the feeding trial	ALIA, UMU, UNITO, EGE, ISA-CM, RAYHANA	07/03/2022
3	Pilot transport and installation	ENTOMO	28/10/2022
4	Workshop training in Tunisia	ISA-CM, RAYHANA, ENTOMO	15/11/2022

No. Item	Actions to be conducted	Responsible partner	Deadline
5	Dry insect production	ENTOMO, ALIA, UMU, UNITO, EGE, ISA-CM, RAYHANA	Continuous

Table 6 shows the planned dry insect larvae which have been produced for the feeding trials up to date

Table 6. Planning for dry larvae supply to the feeding trials

No. Item	Larva production planning	Quantity (kg)	User	Deadline
1	UMU pretesting	0.5	UMU	08/04/2022
2	UNITO pretesting	0.5	UNITO	08/04/2022
3	Feeding trial UNITO	50	UNITO	15/05/2022
4	Feeding trial UMU	150	UMU	15/09/2022

## 6 CONCLUSIONS

The insect selected to carry out the experience will be *Hermetia illucens*, Black Soldier Fly, for its plasticity and production capacity eating different by-products. It is very adaptable, and is present in Europe and in all the Mediterranean area. BSF it is, it is not considered as a pest and do not transmit diseases, actually helps to control some.

It has been defined a production methodology, dividing the production into several stages with nursery/hatchery, breeding, fattening, food gathering, pupae preparation and processing. Those processes will be similar in the IPP. A risk management table is also offered for security and health issues in the IPP.

Locations' characteristics have been described based on the information collected from the SUSTAvianFEED partners in terms of production capacity and climate influence. Furthermore, a description of the pilots in each location is given. Mainly Tunisia ISA-CM as medium scale IPP packed in a shipping container, Rayhana as small house hall pilot not placed in a concrete location but rather as a dissemination activity under Rayhana supervision, and finally ENTOMO IPP in Spain as a representation of highly monitored side where the insect production will be demonstrated and will produce insects for the feeding trials of ALIA, UNITO, and UMU.

A planning of the IPP is given with details of the installation in each area and delivery of insect biomass as long as it is known now.

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## ANNEX I. Data collected from the partners

### 1.1 Turkey

Feedstock available for insects												
Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Vegetable and fruits trims Kg/d	350	350	350	350	350	350	350	350	350	350	350	350
Ketchup and mayonnaise Kg/week	300	300	300	300	300	300	300	300	300	300	300	300
Ready soup Kg/d	300	300	300	300	300	300	300	300	300	300	300	300
Bakery products Kg/d	100	100	100	100	100	100	100	100	100	100	100	100
Grape pomace Kg/month							1000	1000	1000			
Mandarin waste Kg/month										10000	10000	10000

Legislation
<p><b>Legislation:</b> linked with feedstock, every country, have different regulations regarding to insect farming or just lack the regulation. And although we could assume European legislation as a standard, we might lose the potential to exploit the insects widely.</p> <p><b>There are no regulations so far. It is expected that EU legislations will be accepted when the issue comes to the agenda.</b></p>

Climate													
	Jan	Feb	Mar	Apr	May	Jun	Jul	Augo	Se p	Oct	No v	De c	Ave
Max Tem(°C)	12	13	16	21	26	31	33	33	29	24	19	14	23
Min Tem(°C)	5	6	8	11	15	20	22	22	9	15	11	7	14
Max RH (%)													
MIN (%)													
AVERAGE RH, %	79	75	71	66	59	49	42	44	53	68	75	80	63
Hours light	9:30	10	11	11:30	13:50	14:30	14:40	14	13	11	10	9:30	12:10



## Water, Energy and Internet resources

- Type of water available (Urban, agricultural, waste water): **Agricultural**
- Flow:
- Pressure:
- Limitations(time, volume, quality): **No limit**
- pH: **7.53**
- Electroconductivity:**521  $\mu$ S/cm**

### Electricity

- Voltage: **220-260 V, 50-60Hz**
- Type(monophse, bi-phase, tree-phase): **All available**
- Limitations(time, stability, power): **No limit**

### Internet

- Ethernet or wi-fi: **Wi-fi**
- Data flux

## Location

The main company (Germina) was established by 5 medicine doctors in Ankara. The aim of the company is to develop new, environmentally friendly and effective strategies and solutions for sustainable agriculture, organic farming, poultry and aquaculture, and to create models that will facilitate their widespread use.

BSF Germina is a branch of Germina located in a small town in İzmir, about 70 km away from the EGE University campus.

The production area is within a town (Çamlı, Guzelbahce, Izmir).

Total: 200 m<sup>2</sup> area.

Green area: 100 m<sup>2</sup>

Total closed area: 60 m<sup>2</sup> (container)

- Fly mating area: 20 m<sup>2</sup>
- Larva feeding area: 40 m<sup>2</sup>
- Waste management area: 40 m<sup>2</sup>

Currently, BSF Germina gives live larvae to chicken producers. However, there is equipment to perform drying and defatting when necessary.

## Human resources

**The owner of the facility is a medicine doctor (radiation oncologist, retired). He takes care with one worker.**

## Other companies

There are two more producers in İstanbul. These are small and amateur producers. We talked with these producers by phone. They said that, if necessary, the two of them could work together to provide us with larvae.  
There are also producers producing flour worms in our region. These manufacturers are also enthusiastic about their products.

## 1.2 Tunisia

Feedstock available for insects (kg/day)												
Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dic
Bread and Pasta	16	16.	16.5	**	16.5	16.5	**	**	16.5	16.5	16.5	**
Legumes and fruits	17.	17.	17.5	**	17.5	17.5	**	**	17.5	17.5	17.5	**

(\*) canteen wastes were estimated during October and November

(\*\*) During holidays of July, August and mid-April and mid-December the canteen of ISA-CM is closed

## Legislation

No specific regulations regarding insect farming except the DGSVSI (Direction générale de la santé végétale et la sécurité des intrants)-Ministry of Agriculture has to check if the insect is in the quarantine list and if the necessary infrastructures and equipment are available to guarantee the security of the insect farming.  
Some interviewers informed that a study of the impact of the project is probably needed

## Climate

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ave
Max Tem(°C)	12.3	12.9	14.6	17.6	20.0	25.1	27.0	27.2	24.9	21.1	16.44	13.0	19.4
Min Tem(°C)	7.7	7.6	9.2	12.3	14.9	18.5	21.1	21.6	20.0	16.3	11.18	7.8	14.0
Mean Tem (°C)	12.4	12.9	14.6	17.6	20.0	25.1	27.0	27.2	24.9	21.1	16.44	13.0	19.4
Max RH (%)	94,2	96,6	97.2	95	95.8	97	95.6	93.4	95	96.8	98.4	97	96.0
MinRH (%)	28,2	24,8	15	19.2	15.6	18	15	18.6	23.4	15.4	27.4	32	21.0
Hours light	10:00	10:55	11:5	13:06	14:04	14:34	14:22	13:31	12:22	11:17	10:16	09:44	12:40
Rainfal (mm)	7,18	17,38	51,02	23,2	11,1	3,7	0	31,66	130,2	66,4	50	42,9	36,2

## Water, Energy and Internet resources

### Water

- Type of water available (Urban, agricultural, waste water): urban and agricultural
- Flow: 10-12l/m,
- Pressure: 3 bars
- Limitations (time, volume, quality) low flow and frequent water cut-off in summer season and salinity 3g/l
- pH: 7
- Electroconductivity: 3.5g/l

### Electricity

- Voltage: until 250V
- Type (monophase, bi-phase, three-phase): three-phase
- Limitations (time, stability, power): time + stability + power

### Internet

- Ethernet or wi-fi: Only Ethernet in the administration and staff offices and not in the experimental farm
- Data flux

## Location

Please describe: space available(m<sup>2</sup>): free space  
Type of ground: torff  
Non Levelled  
the facilities available: water, electricity, tarmac path

## Human resources

Please: Type of personnel, number and committed time (Students for training periods).

## Other companies

- NextProtein in Grombalia 109km from Sousse
- A Startup of Ahmed Limaiem (Mornag : 132km from Sousse)

### 1.3 Italy

Feedstock available for insects (kg or tonnes, if indicated)												
Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Egg	600	600	600	600	600	600	600	600	600	600	600	600
Beer threshers	28000	28000	28000	28000	28000	28000	28000	12000	12000	28000	28000	28000
Exhausted yeast	800	800	800	800	800	800	800	800	800	800	800	800
Pulp fruit	360 t	360 t	360 t	360 t	360 t	360 t	360 t	360 t	360 t	360 t	360 t	360 t
Whey	300	300	300	300	300	300	300	300	300	300	300	300
Liquid vinasse	20 t	20 t	20 t	20 t	20 t	20 t	20 t	20 t	20 t	20 t	20 t	20 t
Solid vinasse	30 t	30 t	30 t	30 t	30 t	30 t	30 t	30 t	30 t	30 t	30 t	30 t
Carrot peels + other vegetable	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t
Potatoes peels + other vegetable	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t	90 t
Silver film	200	200	200	200	200	200	200	200	200	200	200	200

Legislation
EU Regulation, no additional Regulation by Italian Government

Climate													
	Jan	Feb	Mar	Apr	May	Jun	Jul	Au go	Sep	Oct	Nov	Dec	Ave
Max Tem(°C)	11	15	18	22	26	27	28	28	28	25	20	14	22
Min Tem(°C)	- 5	0	1	4	6	10	15	13	8	4	0	0	5
Mean RH (%)	74	75	66	71	72	75	73	72	75	78	79	79	74
Hours light	9.19	10.3	12.1	13.36	14.58	15.41	15.21	14.9	12.38	11.4	9.4	8.56	12

### Location

Note: Not required to UNITO

### Human resources

Note: Not required to UNITO

### Other companies

Note: Not required to UNITO

## 1.4 Spain

### Feedstock available for insects (Tons/day)

Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Beer bagasse	10	10	10	10	10	10	10	10	10	10	10	10
Legumes Vegetables trims	10	10	10	10	10	10	10	10	10	10	10	10
Whey	2	2	2	2	2	2	2	2	2	2	2	2

### Legislation

EU Regulation, no additional Regulation by Spanish Government

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ave
Max Tem(°C)	13	15	18	20	24	30	33	33	28	23	18	14	22.4
Min Tem(°C)	2	3	5	7	11	15	18	18	15	10	6	3	9.4
Mean Tem (°C)	7	8	11	14	18	23	23	26	21	16	11	8	19.4
Max RH (%)	86.2	75	85.2	86	75	70	65	55	60	75	80	93	75.43

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ave
MinRH (%)	34.2	46.8	49	46	38	35	33	18	24	36	42	67	39.1
Hours light	9.9	10.8	12	13.2	14.3	14.8	14.5	13.6	12.4	11.2	10.1		12
Precipitation (mm)	15.8	18.2	18.8	21.0	17.1	12.1	3.4	6.0	18.1	27.3	23.6	19.4	16.8

### Water, Energy and Internet resources

#### Water

- Type of water available: urban
- Flow: 50l/m,
- Pressure: 3 bars
- Limitations (time, volume, quality): no limitations
- pH: 7
- Electroconductivity: 150µS/cm
- **Electricity**
- Voltage: 250-370V
- Type (monophase, bi-phase, three-phase): three-phase and mono-phase
- Limitations (time, stability, power): No limitations

#### Internet

- Ethernet or wi-fi: Ethernet and Wi-Fi in all the area (lab, offices, processing and production)
- Data flux: 50 mb/s

### Location

Please describe: space available(m<sup>2</sup>): Warehouse 300m<sup>2</sup>, located in industrial area.

### Human resources

Graduated, masters and non-qualified workers

### Other companies

- Bioflytech. Also located in Murcia region
- BioEnto. Located in Madrid Region