

Effects of Larval Density and Feeding Rates on the Bioconversion of Vegetable Waste Using Black Soldier Fly Larvae *Hermetia illucens* (L.), (Diptera: Stratiomyidae)

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Abstract The exploitation of fast degradation organic solid waste through the use of black soldier fly larvae *Hermetia illucens* constitutes a promising alternative in waste management given that it generates several products of added value (animal feed, larval compost, biofuels). The proper development of this process and its application at an industrial scale implies knowledge of the load capacity itself. In this context, with the present work the effects of larval density and feeding rate on the bioconversion of organic solid waste were evaluated. A composite central design was used to obtain response surfaces. The results show that both variables have a significant influence on the bioconversion process, with larval density the most influential element. Ideal conditions were determined, within the experiment's range, to be a larval density of 1.2 larvae/cm² and a feeding rate of 163 mg/larva/day (dry base) which produces up to 1.1 kg/m²/day of larval compost and 59 g/m²/day of larval biomass, on dry base. In order to generate the most quantity of biomass, the process tolerates larval density values of up to 5 larvae/cm² without significant influence on the process as long as it is provided

with a feeding rate no larger than 95 mg/larva/day (dry base).

Keywords *Hermetia illucens* · Larval density · Treatment of organic solid waste · Load capacity · Optimization

Introduction

A variety of factors, both biotic and abiotic, influence the digestion processes of many insects in nature. Knowledge of these factors is of great importance, especially for species with environmental and economic value. This is the case for black soldier fly larvae *Hermetia illucens* (BFSL) which convert the residual proteins of organic waste and other nutrients into a diverse number of products with added value: animal feed [1–3], soil conditioner [4] and biofuels [5–7], thanks to the action of their intestinal microorganisms [8] and enzymes [9], while considerably reducing the potential contamination by 50–60 % [10], with a reduction of nitrogen and other minerals concentration in the range of 40–62 % [11].

The bioconversion process using BFSL is affected by the type, quantity and quality of feed [12] and diverse environment factors. The proper combination of these parameters could notably improve the bioconversion efficiency in terms of reduction of unwanted composites, stabilization of organic matter, optimal production of biomass, bioconversion time, among others. Factors such as lack or excess of feed, which is related to the larval feeding rate, and overcrowding, related to larval density, haven't been studied extensively [13, 14]. Studying these is important given their relation to the system's load capacity, that is, the maximum population size the system can support, while taking into account removable resources such as

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feed and non-removable such as living space [15], these being of increased relevance when one expects to expand the process to a larger scale.

For this reason the goal of the present study was to study the influence of larval density and feeding rate on the bioconversion of organic solid waste.

In Colombia about 25,000 tons of solid waste are generated daily, out of which about 1800 tons/day are disposed of inadequately [16]. The implementation of a treatment and appraisal of rapid degradation organic solid waste (RDOSW), which represent about 60–80 % of all solid waste generated in Colombia [17], would be a promising alternative which would allow to minimize negative effects on the environment especially in low income settings where there is a lack of basic sanitation services.

Materials and Methods

Study Area

The study was performed in the gathering center facilities or Solid Waste Technical Storage Unit (3°22'37.18"N., 76°31'48.60"W) of the Universidad del Valle. The RDOSW were collected from University fruit stands and the Unicentro shopping center.

Colony Establishment

Hermetia illucens pupae were obtained out of captured wild larvae, which were then deposited in a 2.8 × 1.5 × 1.5 m cage, covered with a mesh in order to stop fleeing flies and invasion by parasitoids and predators. For raising the larvae, the methodology of Sheppard et al. [13] was followed, with some modifications.

RDOSW Preparation

The substratum consisted of a mix of vegetal refuse (plantain, potato, cabbage) and fruits (banana, papaya) with

Table 1 Refuse composition

Waste	Dry base (%)	Humidity ^a (%)	Humid base (%)
Plantain	25.99	83.11 ± 1.07	21.49
Potato	27.67	76.88 ± 2.11	16.72
Banana	17.34	87.88 ± 1.98	19.98
Papaya	4.13	90.86 ± 0.02	6.31
Cabbage	24.87	90.22 ± 0.51	35.50
Total	100		100.00

^a Humidity was measured at 60 °C until there was constant weight, with 3 samples collected on separate days

a physical composition as specified in Table 1. This particular substrate was chosen as it represents the refuse generated by the restaurants and fruit stands close to the University.

With the use of a shredder, the waste was reduced to particles between 0.5 and 1 cm in size. The density of the same was determined, per the recommendations of Sakurai [18], aiming to size up the containers to be used in the experimental phase.

Experimental Design

The incidence of the variables on RDOSW bioconversion was analyzed through an experimental composite central design with two factors: LD (Larval Density) and LFR (Larval Feeding Rate) (Table 2). The design includes four star points and five central points for a total of 17 experimental units. This design was chosen since it allows optimizing the response to a given process, through analysis of the response surface.

As response variables the following were used: waste reduction index (Diener et al. 2009) [14] (WRI) (Eq. 2), the relative growth rate (RGR) which is the efficiency of larval growth throughout the experiment [19] (Eq. 3), the temperature change (ΔT), the final pH and the leachate production rate (LPR) which corresponds to the quantity of leachate produced per quantity of provided substrate (Eq. 4). The mass balances were performed on dry base. The humidity was determined by drying in an oven at 60 °C for 24 h, the remaining humidity was measured with a humidity scale Ohaus MB45 set on Fast at 60 °C and automatic shutdown criteria A30 [20].

$$\%R = \frac{S - R}{(S)} \times 100 \quad (1)$$

$$WRI = \%R/t \quad (2)$$

$$RGR = (WL_f - WL_0)/(t_f \times WL_0) \quad (3)$$

$$LPR = (VL_f)/(S) \quad (4)$$

where S is the total quantity of substrate provided throughout the experiment, R is the residue left after bioconversion (non-digested substrate + excretion products), t is bioconversion time, defined as the moment when 50 % of larvae have developed to pre-pupae [14], WL_f is larval

Table 2 Experimental factors

Factor	Description	Low level	High level	Units
A	Larval density	2	6	lv/cm ²
B	Feeding rate	60	200	mg/lv/day

lv larvae

weight by the end of the process (pre-pupae total weight), Wl_0 being larval weight at the start of the process, VL_F the volume of leachate accumulated over the experiment and t_f as final experiment time.

This phase was performed at laboratory scale in containers (larval incubator) of equal surface area (49 cm^2). To allow for evacuation of leachate, an orifice of approximately 2 cm diameter was opened on each incubator. The larval incubators were covered with geotextil to protect against both light and invasion by other insects.

Collection of samples and supplying the larvae with waste was performed following the methods described by Diener et al. [14]. The pH and temperature were measured with the help of a pH meter SoilStik™.

Statistical Analysis

STATGRAPHICS Centurion XV version 15.2.06 software was used to obtain Pareto and response surfaces graphics.

Results and Discussion

Larval Density and Feeding Rate Tracking

A %R between 25 and 45 % was obtained, with bioconversion times of 14–20 days. Final larval weights (Wl_F) between 50 and 72 mg/larva in dry base, temperature difference between 0.5 and 2.9 °C, final pH between 4 and 8 units and leachate production rate (LPR) between 0.3 and 1.8 ml/g of dry base substrate. All of these depending on larval density (LD) and larval feeding rate (LFR) (Table 3). According to these results, one can observe that the best WRI values are obtained with a TAL of 60 mg/larva/day, corresponding to experiments 6, 7 and 8. This is due to the higher quantity of larvae per gram of substrate when compared to higher LFR, which makes it more efficient for waste reduction.

Additionally an increase in bioconversion times was found at high LD present in experiments 1, 2 and 10, due to the higher competition for nourishment, so the biological effect was a prolongation of development time as a physiological consequence of a low feed offer and high larval density. Something similar occurs when the larvae are exposed to a low LFR as in experiment 13. This matches the results obtained by Diener et al. [14], where for a LFR of 12.5 mg/larva/day (60 % humidity), the bioconversion time was prolonged for more than 30 days. It should be highlighted that in the present study’s experiments there was no radical food shortage since the larvae reached weights of over 50 mg, well over the 35 mg required minimum for migration [14].

Table 3 Behavior of %R, t, WRI, WLF, RGR, ΔT and LPR at different LD and LFR

ID	Normalized factors		Response variables									
	LD	LFR	LD (lv/cm ²)	LFR (mg/lv/day)	%R	t (d)	WRI (%/day)	WLF (mg/lv)	RGR (mg/mg/day)	ΔT (°C)	pH	LPR (ml/g)
1	1	0	6	130	31	19.4	2	68.6	2	2.5	4.72	1.8
2	1	1	6	200	30.9	20.3	2	54.5	1	2.5	4.08	1.83
3	0	1	4	200	29.6	17.9	2	71.4	2	2.3	4.01	1.5
4	-1	1	2	200	34	13.9	2	58.1	2	1.4	5.23	0.9
5	-1	0	2	130	35.2	14.3	2	61.1	2	0.9	7.62	0.57
6	-1	-1	2	60	42	16.3	3	56.9	1	0.5	8.03	0.32
7	0	-1	4	60	44.5	17.9	2	60.7	2	0.9	7.62	0.96
8	1	-1	6	60	39.4	15.5	3	55.7	2	1.7	7.5	1.18
9	0	0	4	130	25.2 ± 2.8	16.0 ± 1.6	1.570 ± 0.3	62.3 ± 5.2	1.772 ± 0.16	2.3 ± 0.12	5.47 ± 0.30	1.24 ± 0.08
10	1.41	0	6.8	130	28.6	18.9	2	65.1	1	2.5	4.3	1.79
11	0	1.41	4	228.7	30.1	16.3	2	59.8	2	2.9	5	1.64
12	-1.41	0	1.2	130	31.4	14.2	2	51	2	0.5	7.21	0.4
13	0	-1.41	4	31.3	40.2	19	2	52.1	1	1	7.26	0.34

lv larvae

On the other hand, for high LD and LFR the WRI tends to diminish (Fig. 1a), which suggests that there has been an excess of feed and despite the increase in density, larvae possesses an upper boundary for its consumption. This feed

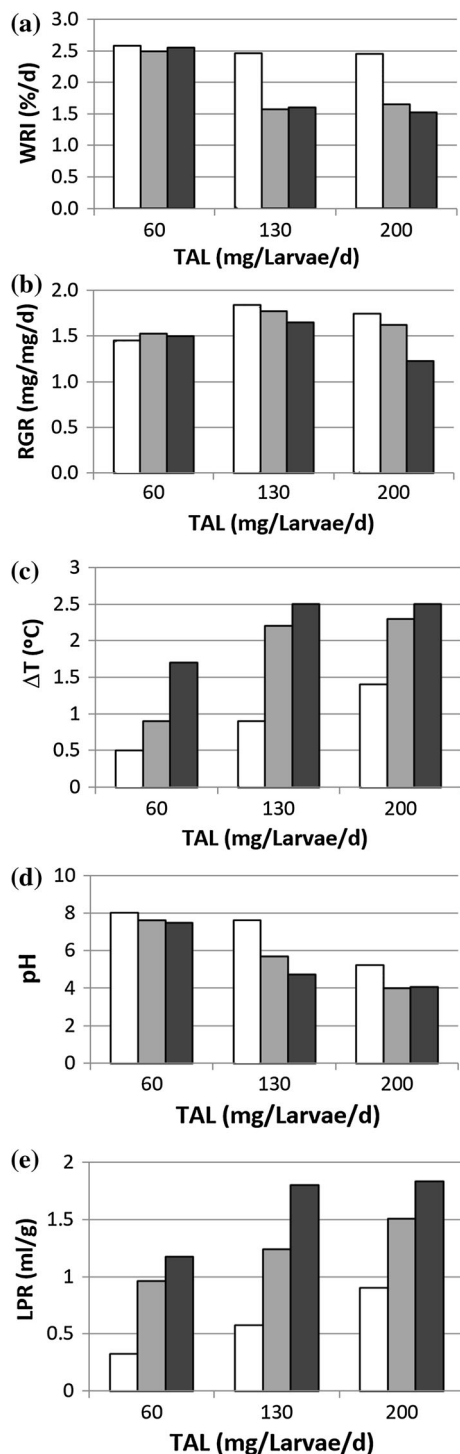


Fig. 1 Bioconversion behavior at different LFR (60, 130 and 200 mg/larva/day) and LD: white bar 2 larvae/cm², light grey bar 4 larvae/cm², dark grey bar 6 larvae/cm²

surplus was observed in qualitative form and by tracking pH (Fig. 1b). All of the experiments at LFR less or equal to 60 mg/larva/day presented pH between 7 and 8, while experiments at LFR greater or equal to 200 mg/larva/day presented pH between 4 and 5. It is well known that these larvae tend to stabilize pH levels [21], however, these results show that in case of excessive feed, anaerobic conditions are created due to the accumulation of undigested feed, which drives pH levels down. This possibly affected their growth as well and therefore the RGR index was reduced (Fig. 1c).

By comparison, the temperature difference with room temperature (average between 26 and 28 °C), tends to increase for experiments with a higher LD (Fig. 1d), due to the larval overcrowding and the heat generated by their movement. These findings confirm those obtained by Olivier [22], where bioconversion managed to reach temperatures over 30 °C. Therefore, for countries that have a weather season, best results are obtained when using high larval density since by saving the self-generated heat it can counteract the low temperatures that have a negative effect on the bioconversion process.

For tropical countries as our own it is recommended to work with LD levels that don't surpass 30 °C, since this is the optimal temperature for bioconversion [21, 23].

Finally, the leachate production is linked not only to the quantity of waste provided and their humidity, but also the LD which contributes to the hydrolysis of the organic matter. The higher these values are, the higher the leachate production will be (Fig. 1e). This is a key factor in the design and implementation of this project at large scale, since adequate drainage depends on the leachate quantity in order to avoid undesirable conditions for the process, and for its final disposal or treatment afterwards.

Statistical Analysis

Performing statistical analysis of the reduction index (WRI), $R^2 = 78 \%$, shows that the independent variables LD and LFR (sorted in descending order of significance) have a negative influence over the WRI ($p < 0.05$). The best combinations to work with in order to increase this index, within the experiment's range, are then either at low LD between 1.2 and 3 larvae/cm² (−1.41 and −0.6, normalized data) and high LFR between 130 and 230 mg/larva/day (0 and 1.4, normalized data) or at high LD between 5 and 7 larvae/cm² (0.6 and 1.41, normalized data) and low LFR between 30 and 60 mg/larva/day (−1.41 and −1, normalized data) (Fig. 2a).

Statistical analysis of the relative growth rate (RGR), $R^2 = 86 \%$, shows that only the larval density (LD) influences negatively the RGR ($p < 0.05$). Since as LD rises the larvae competition for food increases, and

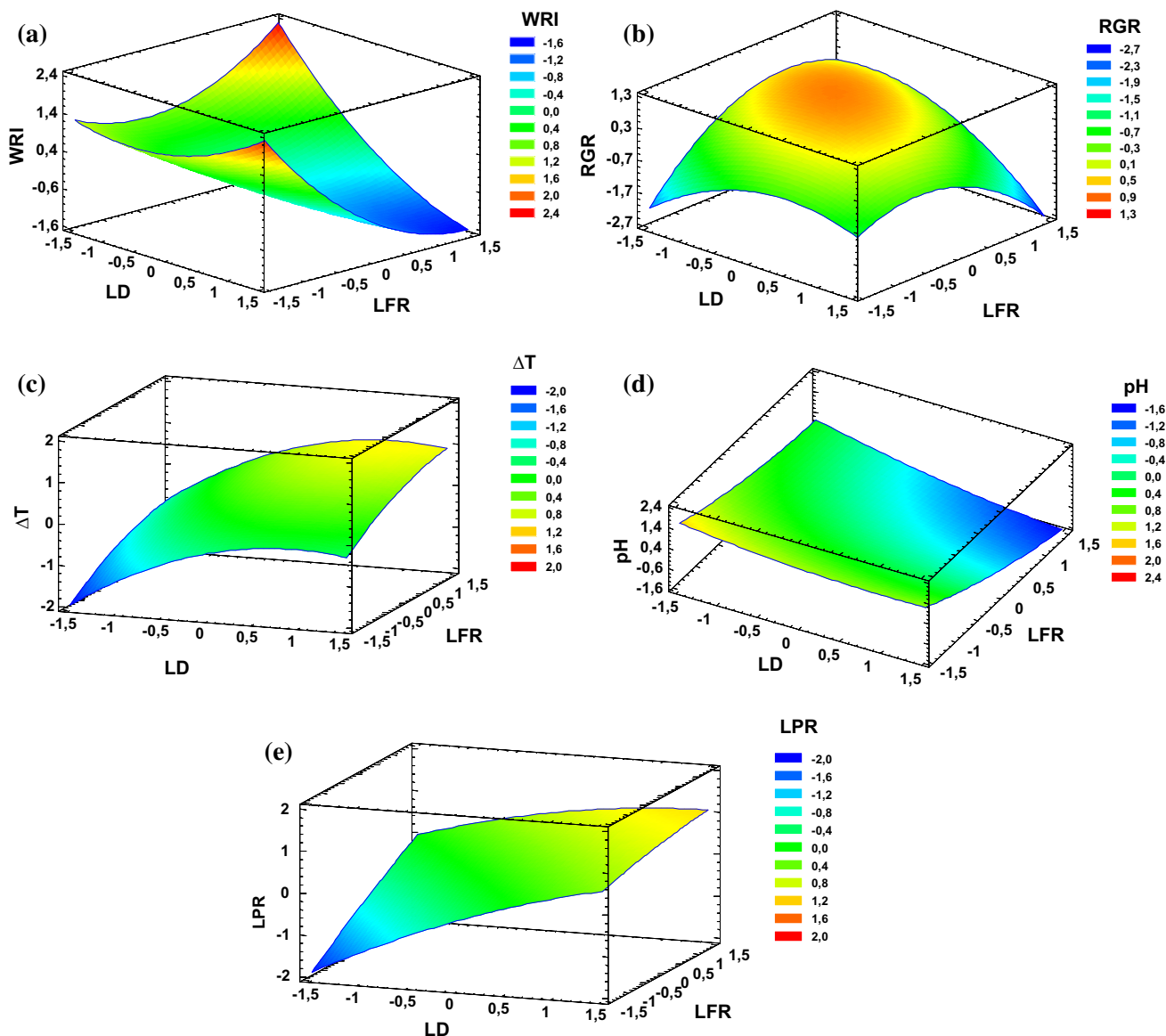


Fig. 2 Response surfaces

therefore their growth weight is affected. Additionally, the LFR–LFR and LD–LD interactions had significant negative effects, ($p < 0.05$, 0.0019 and 0.0077 respectively) which indicates that the RGR diminishes beyond specific values for LFR and LD, which can be found among the extreme values of these variables. This means that there are optimal points for LFR and LD that are conducive to maximizing the RGR value (Fig. 2b).

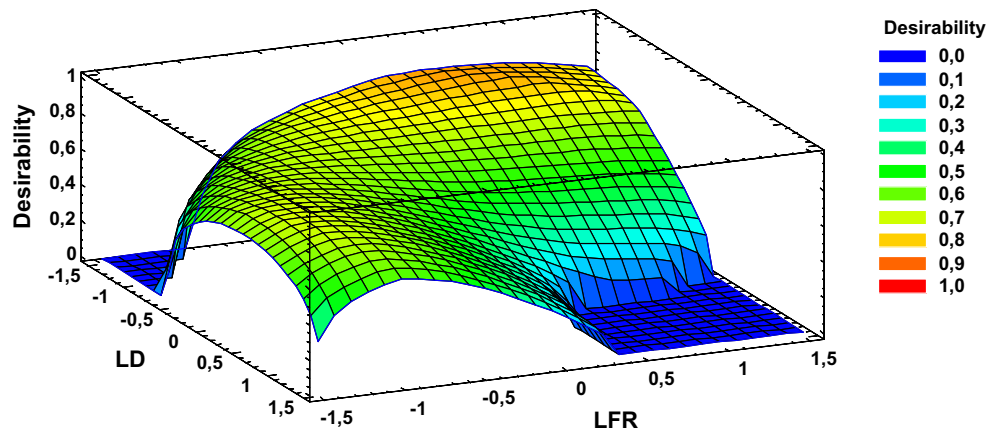
Statistical analysis of the temperature difference (ΔT), $R^2 = 93\%$, shows that both variables (LD and LFR) have a positive influence over the temperature difference, being LD the most influential. The higher these values are, the higher the system temperature increase will be (Fig. 2c).

Taking this into account it is very important to not surpass the overcrowding temperature.

As for pH, the statistical analysis, $R^2 = 85\%$, shows that both variables (LD and LFR) have a negative influence, which leads us to say that as these values go higher, the pH value within the system will lower. Therefore the best pH values are obtained at lower LD and LFR (Fig. 2d).

Statistical analysis of the leachate production (LPR), $R^2 = 95\%$, shows that both variables (LD and LFR) have a positive influence over the production of leachate. The higher these values are, higher quantities of leachate will be produced (Fig. 2e).

Fig. 3 Estimated response surface for optimization of multiple response variables



Therefore one could state that the undesirable zones to perform the bioconversion process are present at high LD and high LFR, which doesn't favor any of the response variables since it tends to diminish the values for WRI, RGR and pH while increasing ΔT and LPR. The rise of these last two variables is undesirable in our context since it is linked to high temperature increase and high leachate production.

Optimization of Multiple Response Variables

The combination of factors that optimize the response variables (desirability function) is found at low levels of LD (between -1.5 and 0 , normalized data) and medium values of LFR (between 0 and 1) (Fig. 3).

The best estimated parameter combination, within the range of the experiment, was: LD of 1.2 larvae/cm² and LFR of 163 mg/larva/day (dry base) approximately, which, according to the optimization model, would achieve a WRI of 2.55 %/day, RGR of 1.70 mg/mg/day, ΔT of 0.7 °C, final pH of 6.9 units and a LPR of 0.5 ml/g.

Under these conditions, assuming a bioconversion time of 17 days, it would produce approximately 59 g/m²/day of biomass, in dry base, a far lower value than reported by Diener et al. [14] (145 g/m²/day). This is due to this work using a LD of 5 larvae/cm², a value higher than the one used by Diener (2 larvae/cm²).

This leads us to state that if the main goal of bioconversion is obtaining biomass, then working with high LD and slightly risking the other response variables is a must, as long as the overcrowding limits are not surpassed.

Using the regression mode, granted by the statistical analysis, assuming a LD of 5 larvae/cm² and a LFR, in dry base, of 40 mg/larva/day, one would obtain a WRI of 2.5 %/day, RGR of 1.48 mg/mg/day, ΔT of 1.5 °C, final pH of 7.4 units and a LPR of 1 ml/g. It is noticeable how

the RGR index diminishes, however, being this a higher density a larger amount of biomass will be obtained (158 g/m²/day). It bears highlighting that ΔT and the leachate quantity increases, which should also be taken into account when it comes to deciding to work or not at high LD.

Conclusions

The studied variables (LD and LFR) presented significant effects ($p < 0.05$) over the response variables, being LD the most influential. This is excepting the effect on pH where LFR was more significant.

It is counterproductive for the process to work with both high LD and high LFR (over 5 larvae/cm² and 95 mg/larva/day respectively). These conditions reduce the efficiency of the process, diminishing the WRI and RGR indexes, while reaching acidity values below pH 6 . Additionally it generates higher increases in temperature and leachate production, which, considering the weather conditions of tropical nations, is unfavorable.

If the main goal of this process is obtaining biomass, it is feasible to work at densities between 1.2 and 5 larvae/cm² and feeding rates between 95 and 163 mg/larva/day. Even though ideal conditions, within the range of the experiment, are present at low densities (between 1 and 2 larvae/cm²).

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