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Diapause disruption in *Cirina butyrospermi* Vuillet (Lepidoptera, Attacidae), the shea caterpillar, in Burkina Faso

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RESEARCH ARTICLE

Abstract

The shea caterpillar *Cirina butyrospermi* is an important insect, highly valued as a human food item in Burkina Faso. However, its appearance is seasonal due to its univoltine cycle. This study therefore investigated the possibilities of breaking the nymphal diapause by changing the environmental factors and through the hormonal treatment of prepupae and pupae using bovine insulin and 20-hydroxyecdysone. Changes in humidity and temperature did not result in emergence, suggesting a mandatory nature of the diapause in *C. butyrospermi*. Injection of 20-hydroxyecdysone between 20 and 40 ng on 20 *C. butyrospermi* pupae resulted in 15.24 and 47.5% emergence, respectively. The incubation time varied between 40 and 38 days, respectively. No emergence was observed with the injection of bovine insulin. Dipping of *C. butyrospermi* larvae and pupae in solutions of 20-hydroxyecdysone resulted in similar rates of emergence between the two stages, with slight variations between individual doses: (1) for larvae, emergence was recorded at 10, 8, 5 and 15 mg/l with 98.5, 62.14, 25.73 and 24.16%, respectively; the incubation times varied from 39 days at 5 mg/l to 26 days at 20 mg/l; and (2) for pupae, emergence occurred between 5 and 20 mg/l, with the highest emergence rate recorded at 10, 8 and 15 mg/l with 94.58, 65.83 and 29.58%, respectively; the incubation times varied from 53 days for the lowest dose (5 mg/l) to 37 days (20 mg/l); the best emergence rate of 94.58% coincided with an incubation time of 43 days at 10 mg/l. No emergence was observed beyond 20 mg/l in both stages. Hormonal treatment with 20-hydroxyecdysone did not affect the fecundity of *C. butyrospermi*, with the fecundity of artificially emerging adults overlapping with that of naturally emerging adults. The emergence rate for both was similar. These results contribute to a better understanding of the physiology of this insect, constituting a breakthrough in its sustainable use as human food.

Keywords: *Cirina*, univoltine, moulting hormone, Burkina Faso

1. Introduction

The *Cirina butyrospermi* Vuillet caterpillar, commonly known as *shitumu*, is a valuable protein source for human consumption, particularly for the 'Bobo' ethnic group in Burkina Faso and elsewhere. Caterpillars can be dried, fried or boiled and used in various meals. The insect has exceptional nutritional characteristics, with 63% protein, 15% fat, as well as vitamins and minerals (Anvo *et al.*, 2016b). It also has potential as an ingredient in fish feed (Anvo *et al.*, 2016a). Despite its importance as food, feed and even as a bioconverter (Coulibaly *et al.*, 2016), no attempts have been made at mass rearing for large-scale production.

C. butyrospermi is mainly found in the western and south-western regions of Burkina Faso and neighbouring countries, differing from *Cirina forda* (Testout, 1939). However, its distribution in Burkina Faso is not homogeneous, as it is totally absent in the provinces of Bougouriba and Poni, despite the presence of the shea tree *Butyrospermum paradoxum* Gaertn., known as *karité*. The larvae have been absent from the Central Plateau since 1983, as well as from the provinces of Mouhoun and Kossi (Ouédraogo, 1993).

C. butyrospermi has an annual lifecycle with a pupal diapause that lasts for 10 to 11 months. Basically, the lifecycle comprises a larval, a nymphal and an adult stage. Adults appear at the beginning of the rainy season and lay billions of eggs on the host plant. Eggs hatch on the leaves, where they thrive and increase considerably in weight before moving to the soil for pupation.

In 2012, the laboratory involved in this study embarked on a research programme with the aim of disrupting the diapause to allow the insect to be reared throughout the year. In the world of Lepidoptera, pupal diapause is commonly observed (Denlinger, 2002). It is characterised by the interruption of ecdysone production as a consequence of the lack of prothoracicotropic hormone (PTTH) secretion by the brain. It was therefore assumed that diapause could be logically terminated by adequate injections of ecdysone or PTTH. Previous research also reported that the use of moulting hormone, 20-hydroxyecdysone, bovine insulin and analogues of 'small PTTH' on *Pieris brassicae* pupae successfully terminated the diapause (Arpagaus, 1987; Arpagaus *et al.*, 1986). Hence, based on these observations, it is legitimate to apply a similar procedure to break the diapause in *C. butyrospermi*.

The objective of this research is primarily to identify a mechanism to break the obligatory diapause in *C. butyrospermi* using environmental change or moulting hormone. The study eventually evaluated the effect of hormonal treatment on the fecundity and emergence of *C. butyrospermi* progeny.

2. Materials and methods

Insects

C. butyrospermi eggs were collected on the *Butyrospermum* tree and reared in the laboratory (Supplementary Material Figure S1). The development of *C. butyrospermi* includes five larval instars (respective duration of 4-5 days, 6-7 days, 6-7 days, 4-5 days, and 9-14 days). Thus, the overall larval development lasts between 29 and 38 days under laboratory conditions (temperature: 29±1 °C; relative humidity: 60±5%; and 12L:12D photoperiod). The mean pupal weight is 9.28±0.37 g.

Effect of photophase and temperature on the entry of *C. butyrospermi* into diapause

In 2014, preliminary studies were conducted under laboratory condition to understand the nature of the diapause. These studies evaluated the effect of conventional factors, photoperiod and temperature on the diapause of *C. butyrospermi*. One batch of pupae was placed under standard conditions as mentioned above (photoperiod 12:12; humidity at 80% simulating the onset of rainfall; temperature of 26±1 °C, which is the optimal temperature of growth using an air-conditioned), while another batch of pupae was placed under non-controlled temperature and photoperiod. The humidity of the ambient air of the room was approximately an average of 35% (fluctuation 30-40%).

Effect of hormones on breaking the *C. butyrospermi* diapause

Given the impossibility of breaking the diapause of the insect by involving environmental factors, the study assessed injections of pupae with two hormones, namely bovine insulin and the insect moulting hormone 20-hydroxyecdysone.

The first hormone used in the experiment, namely bovine insulin, was purchased from the firm SANOFI (Gentilly Cedex, France). The second hormone, namely 20-hydroxyecdysone (95% purity, extracted from *Cyanotis vaga*) was purchased from Changzou Dahua Imp. and Exp. Corp. Ltd (Changzhou Jiangsu, China P.R.). The hormone was diluted in 45 ml of distilled water 5 ml of alcohol 100%.

Breaking of the diapause using injections of bovine insulin and 20-hydroxyecdysone

In this study, 20 diapausing pupae were injected with increasing doses (10, 15, 20, 25, 30, 35, 40, 45 and 50 µl/pupa) of a solution of bovine insulin (BioReagent) at a concentration of 10 mg/ml, i.e. ≥250 U.I./ml, used without dilution. Alternatively, diapausing pupae (taken during the first days after pupal moult) were injected with increasing doses (10, 15, 20, 25, 30, 35, 40, 45 and 50 µl/pupa) of the solution of 0.05 mg 20-hydroxyecdysone diluted in 45 ml of distilled water +5 ml absolute ethanol (hence doses ranging between 10 and 50 ng/pupa). The injections were performed laterally at an intersegmental abdominal membrane, according to (Beydon and Lafont, 1983). From the second abdominal segment, the needle was inserted tangentially into the body to avoid intestinal tract injury. The experiment was repeated four times, hence a total of 80 pupae being used per dose.

Breaking of the diapause by the dipping of larvae and pupae in 20-hydroxyecdysone solutions

Since injections of bovine insulin did not result in emergence, the second experiment focused mainly on 20-hydroxyecdysone. Larvae and pupae were dipped in solutions containing 20-hydroxyecdysone, according to the 'Chilo dipping test' initially developed by Sato *et al.* (1968). Increasing doses of the hormone (3, 5, 8, 10, 15, 20 and 25 mg of hormone dissolved per litre of distilled water) were used. Dipping was concerned with the pupae or fifth instar larvae ready to pupate; the pupal moult took place within 24 hours for almost all the treated larvae.

Larvae or pupae were dipped for five seconds in solutions of 20-hydroxyecdysone, then transferred into boxes containing sterile sand. Fifty 5th instar larvae or 50 pupae were used for each of four replicates for each hormone concentration. The injected or dipped pupae were grouped in boxes and were then placed in sand-containing cages for the recording of adult emergence and adult fertility.

Statistical analyses

The Statistical Analysis System, SAS, was used for data analysis (SAS/STAT, 2010; SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was used to test the effect of treatments on the variables measured at a significance level $P \leq 0.05$ for normally distributed data with a balanced device. Where the analysis indicated significant effects, the Student Newman Keuls test was applied to separate averages at level $\alpha=0.05$. All the means are given with the standard deviation.

3. Results

Effect of photophase and temperature on the breaking of diapause in *C. butyrospermi*

The results of this study show that changing moisture conditions, temperature and illumination have no effect in terms of breaking the diapause, as no emergence was recorded. This confirmed the obligatory nature of diapause in *C. butyrospermi* and the challenges when it comes to preventing the diapause (Table 1).

Effect of hormones on the breaking of *C. butyrospermi* diapause

Effect of bovine insulin injections on the breaking of pupal diapause

The injection of *C. butyrospermi* pupae with various doses of bovine insulin (2.5 to 12.5 U.I. of insulin) failed to induce emergence after three months (Table 2).

Effect of 20-hydroxyecdysone injections on the breaking of pupal diapause

Unlike the treatment with bovine insulin, injections with increasing doses of 20-hydroxyecdysone resulted in emergence (Figure 1). Analyses of variance indicated a highly significant difference in the dose effect ($P<0.0004$). Pupal emergence was observed at 20, 30 and 40 ng with respective rates of 15.24, 20.15 and 47.5%. The longest incubation period was recorded at 20 ng (40 days), whereas at 40 ng the incubation time was 38 days (Figure 1). No emergence was observed below 20 ng and above 40 ng.

Breaking diapause by dipping C. butyrospermi larvae in 20-hydroxyecdysone solutions

The dipping of *C. butyrospermi* larvae showed significant differences between the concentrations ($P<0.0001$). Emergence was observed between 5 and 20 mg/l. The highest emergence rates were recorded at 10, 8, 5 and 15 mg/l at 98.5, 62.14, 25.73 and 24.16%, respectively.

Table 1. Effect of photophase, temperature on the emergence of *Cirina butyrospermi*.

Incubation date	Number of pupae	End of incubation	Number of emerging adult	% emergence
02/01/2014	20	30/04/2014	0	0
	20		0	0
	20		0	0
	20		0	0
	20		0	0
Total	100	–	0	0

Table 2. Effect the injection of bovine insulin on pupae of *Cirina butyrospermi*.

Incubation date	Number of pupae	End of incubation	Number of emerging adult	% emergence
02/01/2014	20	30/04/2014	0	0
	20		0	0
	20		0	0
	20		0	0
	20		0	0
Total	100	–	0	0

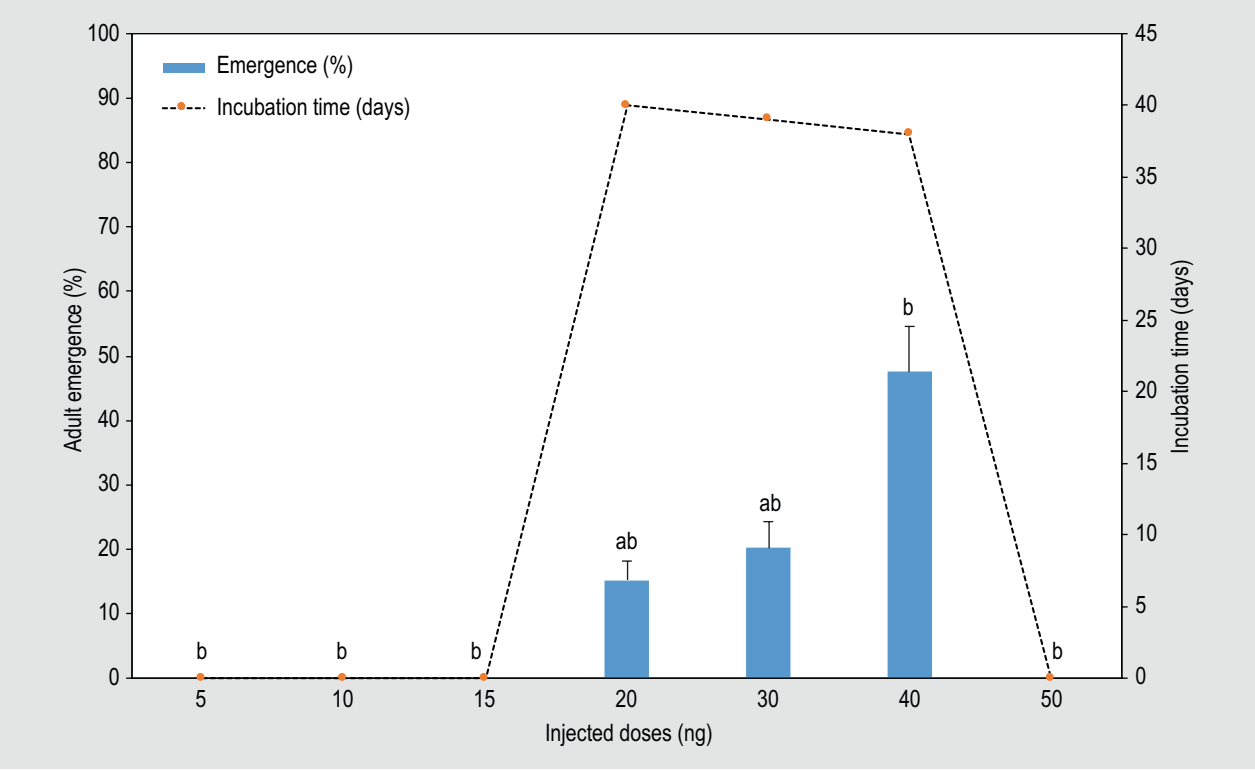

Figure 1. Effect of different doses of 20-hydroxyecdysone on breaking the diapause of *Cirina butyrospermi* (the same alphabetical letters indicate that there is no significant difference between the moisture conditions, n=50, vertical bars are the standard deviation values).

The incubation times varied from 39 days at 5 mg/l to 26 days at 20 mg/l. However, the highest emergence rate, observed at 10 mg/l, was obtained after 30 days (Figure 2). No emergence was observed at 25 and 30 mg/l.

Breaking diapause by dipping C. butyrospermi pupae in 20-hydroxyecdysone solutions

The dipping of *C. butyrospermi* pupae resulted in emergence at between 5 and 20 mg/l, with the highest rate recorded at 10, 8 and 15 mg/l at 94.58, 65.83 and 29.58%, respectively (Figure 3). Statistical analysis indicates a highly significant difference between the different concentrations used ($P<0.0001$). The incubation time varied from 53 days for the lowest dose (5 mg/l) to 37 days (20 mg/l). For the best emergence rate, 94.58%, the incubation time was 43 days at 10 mg/l. Here again, no emergence was recorded above 20 mg/l.

4. Discussion

The lack of emergence observed in the first part of the study suggests a mandatory diapause. Nymphal diapause in *C. butyrospermi* could be considered as a response to a less intense seasonality found in the subtropical regions compared to temperate or Polar Regions (Masaki, 1961; Young, 1982). However, even in tropical savannah regions, harsh and seasonal climatic variations can trigger obligatory diapause. The lifecycle of *C. butyrospermi* cannot be separated from the phenology and the seasonal rhythm of the shea tree. Therefore, the timing of diapause in *C. butyrospermi* must coincide with the period of food availability, considering that the shea tree is the primary host. It could be speculated that considering the seasonal pattern in terms of rainfall and temperature in Burkina Faso and the shea butter tree ecology in general, with distinct rainy summers and dry winters, variety in seasonal patterns is expected to be rare compared to wet tropical areas as discussed by Kishimoto-Yamada and Itioka (2015). This may account for the mandatory diapause in *C. butyrospermi*.

Photoperiod is also considered as dominant diapause-inducing cue and may not vary even in the context of climate change (Bale and Hayward, 2010). Although, higher temperatures may modify normal rates of development, leading to a physiological asynchrony, the shift that maybe required in disrupting diapause might require long term dataset (Bale and Hayward, 2010).

Diapause in any postembryonic stage is generally considered to be caused by the absence of one or more growth-controlling hormones. It could therefore be terminated once endocrine function has been restored (Beck, 1980). *C. butyrospermi* did not respond to injections of bovine

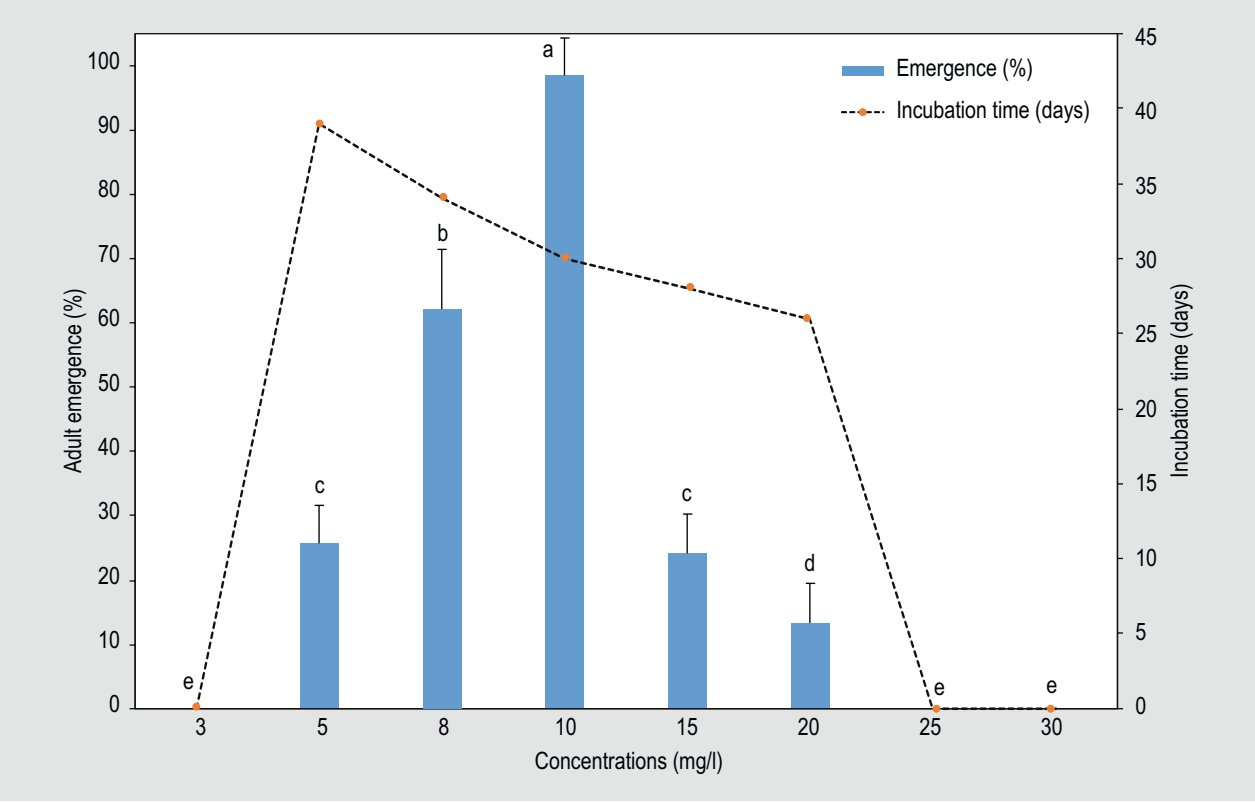

Figure 2. Effects of larval dipping of *Cirina butyrospermi* in solutions of 20-hydroxyecdysone on preventing diapause (the same alphabetical letters indicate that there is no significant difference between the moisture conditions, n=50, vertical bars are the standard deviation values).

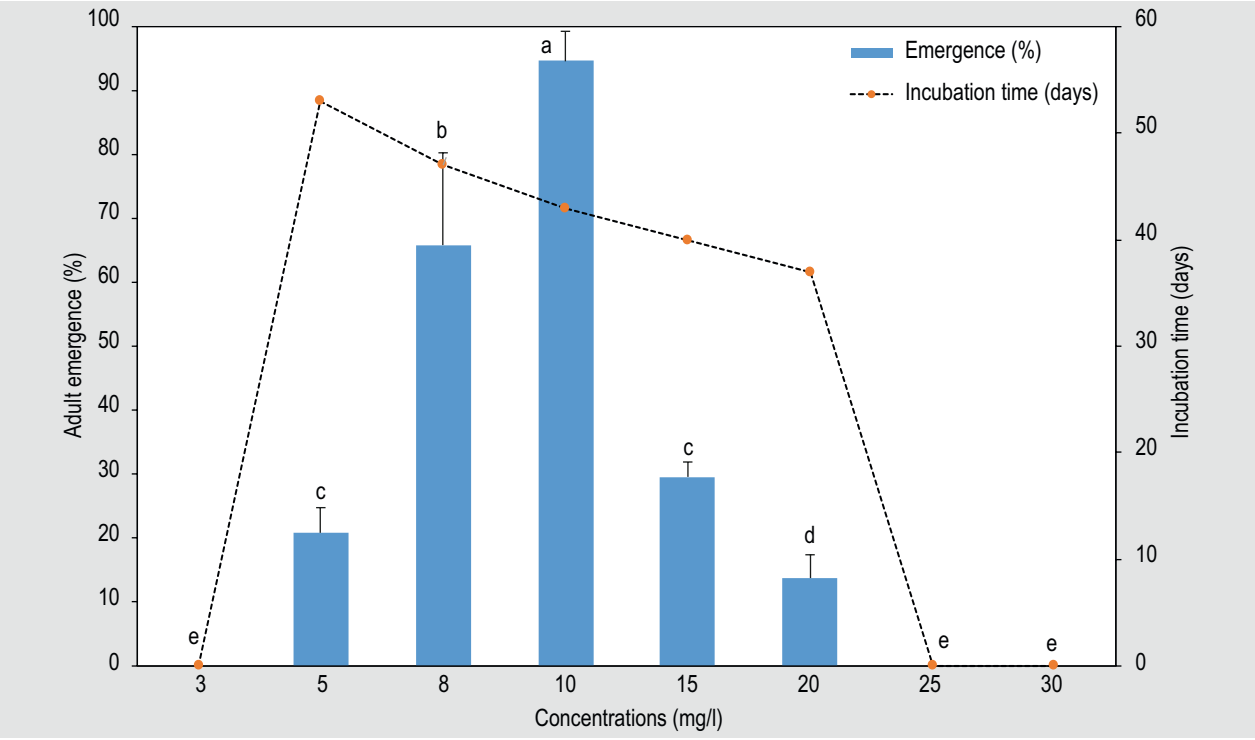

Figure 3. Effects of dipping pupae *Cirina butyrospermi* in solutions of 20-hydroxyecdysone on breaking diapause (the same alphabetical letters indicate that there is no significant difference between the moisture conditions, n=50, vertical bars are the standard deviation values).

insulin. This particular work differs from the findings of Arpagaus (1987), who disrupted diapause in *P. brassicae* 22 days after the injection of pupae at 100-200 µg with bovine insulin. Although this study used a broader range (100 and 500 µg of insulin), no emergence was observed. Noteworthy is the facultative character of *P. brassicae* diapause.

Our findings are different from that described for *Antheraea mylitta* Drury where injections with with ox pancreas insulin elicited response in the diapause physiology of that lepidopteran Saturnidae (Sinha *et al.*, 1993). The difference in findings could be ascribed to the lower range of dosage used compared to that in our study. This requires further investigations.

On the other hand, the injection of 20-hydroxyecdysone in *C. butyrospermi* provoked pupal emergence. These results corroborate the findings with regard to *Graellsia isabelae* Oberthür (OPIE, 1998; Ylla and Bellés, 1992).

Considering the narrow range of active concentrations and the low doses used, it is hypothesised that the treatments used in this study only reactivate the brain-prothoracic gland axis. This might have triggered the later endogenous production of higher amounts of ecdysteroids, which allow for pupal-adult development, as described in respect of *P. brassicae* (Arpagaus *et al.*, 1986; Beck, 1980).

In the dipping experiments, results also showed emergence to be dose-dependent. Although the emergence obtained between larvae and pupae appeared similar overall, comparing doses individually resulted in slight differences. For instance, at 10 mg/l 20-hydroxyecdysone, emergence was 98.5% for larvae, compared to 94.58% for pupae, whereas at 8 mg/l 20-hydroxyecdysone, the rate was 62.14% for larvae compared to 65.83% for pupae. The higher rates of emergence obtained in the 'Chilo dipping test', within a narrow range of concentrations, could be explained by sustained hormone levels, which enhanced cuticle permeability. Several studies have revealed water to be an adequate solvent for the hormone 20-hydroxyecdysone (Hasegawa and Ata, 1971, 1972; Robbins *et al.*, 1968; Sato *et al.*, 1968; Sehnal, 1972). In this particular case, water solutions of 20-hydroxyecdysone proved highly effective on both pre-pupae and pupae. However, the study did not allow for speculation on whether the hormone penetrated throughout the body surface or preferentially through the stigmates. In any case, this highlights the importance of identifying the adequate dose to be used to achieve higher emergence rates. From a rearing point of view, the fecundity of artificially emerging *C. butyrospermi* adults is 437±96 eggs (range 390-636), overlapping with naturally emerged adults (485±127 eggs; range 329-729). The mean hatching rate for both was 91.48±2.14%. Therefore, hormonal treatment with 20-hydroxyecdysone did not significantly affect the fecundity of *C. butyrospermi*.

The season of *C. butyrospermi* occurs once per year in Burkina Faso, and the waiting period for fresh caterpillars is 11 months. Caterpillars are collected from the wild before being processed and dried. This study suggests the possibility of the continuous rearing and supply of fresh shea caterpillars at any point of time in the year. The dipping technique on 20-hydroxyecdysone solution is effective both for larvae and pupae with the same emergence rates. The technique seems to have no effect on the fecundity of the moth. It therefore represents a breakthrough for the thousands of businessmen and -women who are involved in the trade of this insect in West Africa. These results open up new perspectives in the food sector and for entrepreneurship in the country and the region.

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Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2017.0068.

Figure S1. Illustration of the different stages of *Cirina butyrospermi* in Burkina Faso.

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