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Comparison of artificial diets for rearing *Bracon hebetor* Say (Hymenoptera: Braconidae)

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Abstract

The ectoparasitoid *Bracon hebetor* Say is an insect frequently found in storage facilities, where it attacks stored grain pests. The biology of this parasitoid was studied when reared on seven different artificial diets (in vitro rearing), under controlled temperature (25 ± 2 °C), relative humidity ($60 \pm 10\%$), and photoperiod (14-h photophase), and compared to its biology on its natural host *Anagasta kuehniella* (Zeller) (in vivo rearing). The artificial diet contained 60% holotissue of *Diatraea saccharalis* (Fabricius) pupae, 12% fetal bovine serum, 12% lactoalbumin hydrolysate, and 16% egg yolk, enabled development similar to that obtained on the natural host. The life cycle duration (egg–adult) was not significantly different, and the adults reared on this diet promptly paralyzed and parasitized the natural host, though at a lower proportion than those reared in vivo. There was no difference in the longevity of females obtained with these two different rearing systems (in vivo and in vitro). However, about 60% of the larvae developed on the diet failed to produce a protective cocoon during the pupal phase, indicating a sub-optimal quality associated with this artificial medium.

Keywords: Biological Control; In vitro rearing; Ectoparasitoid; Protective cocoon

1. Introduction

Among the countries that initiated research on rearing *Trichogramma* in vitro during the 1970s, only China was able to develop large-scale production by using large silk worm rearings, allowing the release of these insects in large fields to control different pests. Countries such as France, the USA, and, recently, Brazil have also been trying to develop and improve this new rearing technique, which would result in important advances in the present system of parasitoid rearing (Grenier, 1997).

The importance of parasitoids as biological control agents has aroused an interest in dietary studies, viewing mass rearing on artificial media. In vitro rearing techniques have been used to determine the quantitative and qualitative needs, as well as the nutritional biochemistry of natural enemies. However, improvement of these techniques depends on knowledge and understanding of the physiology, nutrition, genetics, and behavior of the parasitoid. Despite these limitations, rearing technologies are available and many parasitoids can be continuously reared on artificial media (Cônsoli and Parra, 1999, 2002; Guerra et al., 1993; Thompson, 1986, 1999; Thompson and Hagen, 1999). Cônsoli and Parra (1997a) reported that 71 species of parasitoids have already been reared on artificial media, with most research concentrated on Diptera and Hymenoptera. A great majority of these papers concern in vitro rearing of endoparasitoids, such as *Trichogramma* spp. in unspecific diets, usually containing some sort of insect element. Fewer papers exist on the rearing of ectoparasitoids on artificial media, mostly involving rearing of *Exeristes roborator* (Fabricius), *Bracon mellitor* Say, *Catolaccus grandis* Burks, *Bracon hebetor* Say e *Diapetimorpha introita* (Cresson) (Carpenter and Greany, 1998; Carpenter et al., 2001; Guerra and Martinez, 1994; Guerra et al., 1993; Thompson, 1975; Xie et al., 1989; Yazlovetsky et al., 1992). Yazgan and House (1970) reported that *Itoplectis conquisitor* (Say) was the first ectoparasitoid reared under chemically defined artificial diet.

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Bracon hebetor is an ectoparasitoid of the larvae of many Lepidopteran pest species that attack stored grain (Brower and Press, 1990). Because it is highly aggressive, this ectoparasitoid is considered to have potential for biological control (Keever et al., 1985, 1986; Prozell and Schöller, 1998). Details on its growth from egg to adult on artificial medium encapsulated in parafilm were reported by Xie et al. (1989). Yazlovetsky et al. (1992) studied the possibility of rearing *B. hebetor* on oligidic medium. They found that it could be reared from the second instar to the pupal stage, obtaining about 80% of normal adults. *B. mellitor* and *C. grandis* have been reared on artificial media with no insect components. Resulting adults exhibited with high viability and were significantly larger that those reared in vivo. Developmental times (egg–adult) similar to those on the natural host were recorded (Guerra and Martinez, 1994; Guerra et al., 1993; Rojas et al., 1996). These authors indicated that the insects reared in vitro had no changes in behavior, fecundity, and fertility compared to those reared on the natural host.

The objective of this study was to adapt a rearing technique and a diet for in vitro production of *B. hebetor*, capable of promoting growth and development, with a quality similar to that of individuals reared on natural host. Such a technique could serve as a "model" system for rearing ectoparasitoids on artificial media, and facilitate studies on the physiology of this ectoparasitoid.

2. Materials and methods

Biological studies of *B. hebetor* were conducted at the Laboratório de Biologia de Insetos of the Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), of the Universidade de São Paulo (USP), in Piracicaba/SP. Parasitoid colonies and experiments were held at 25 ± 2 °C, $60 \pm 10\%$ relative humidity, and a 14:10 (L:D) h photoperiod. The *B. hebetor* stock culture was maintained on its natural host, *Anagasta kuehniella* (Zeller).

The artificial diet for rearing *B. hebetor* in vitro was made with *A. kuehniella* pupal holotissue and holotissue of *Diatraea saccharalis* (Fabricius), which was adequate for the production of large pupae and provided good results for in vivo rearing of this braconid under laboratory conditions (Magro and Parra, 2001). Different quantities of other nutrients were added to prepare seven different diets (Table 1). The diets were prepared in a laminar flow chamber, to maintain aseptic conditions.

To remove the pupal holotissues, 24–48 h pupae of *A. kuehniella* and *D. saccharalis*, reared on an artificial diet prepared according to Parra (1997) and Parra and Mishfeldt (1992), respectively, were subjected to thermal shock (60–62 °C hot water for 10 min) to prevent melanization of the hemolymph by phenyloxidases activity. Following this, pupal surfaces were sterilized in 2% sodium hypochlorite solution for 10 min and then washed twice with distilled water. After this treatment, pupae were placed inside a 15 ml disposable syringe and squashed with a plunger. The liquid from this maceration was collected into an appropriate container and centrifuged at 2000 rpm for 3 min. The precipitate was discarded and only the supernatant was used, either immediately or after storage at −18 °C, until diets were prepared.

Diets were offered to the parasitoids in plastic petri dishes (2.0×1.0 cm), and 150 µl of medium was placed between two sheets of stretched Parafilm. Around 20 *B. hebetor* eggs, obtained from parasitized *A. kuehniella*, were transferred to the parafilm of each plate with a fine tipped paintbrush. These rearing chambers were placed

Table 1
Percentage composition of various components of artificial diets tested for in vitro rearing of *Bracon hebetor*

	Diets						
	1[a]	2	3	4	5	6	7[b]
Pupal holotissues of *D. saccharalis*	60	50	50	—	—	—	40
Pupal holotissues of *A. kuehniella*	—	—	—	60	50	50	—
Fetal bovine serum	12	15	10	12	15	10	—
Lactoalbumin hydrolysate	12	15	10	12	15	10	—
Yeast extract solution	—	—	10	—	—	10	—
Egg yolk	16	20	20	16	20	20	20
Partially skimmed milk	—	—	—	—	—	—	20
Distilled water	—	—	—	—	—	—	10
Neisenheimer[c] salt mixture	—	—	—	—	—	—	10
Antibiotics[d]	0.6	0.6	0.6	0.6	0.6	0.6	0.6

[a] In vitro rearing diet for *Trichogramma galloi* and *T. pretiosum* (Cônsoli and Parra, 1997a).
[b] Diet developed in France for in vitro rearing of *Trichogramma* spp. (Gomes, personal communication).
[c] Formulation [(7.5 g NaCl; 0.1 g KCl; and 0.2 g $NaHCO_3$)/liter of distilled water].
[d] Penicillin, streptomycin, and anphotericin (antibiotic–antimycotic Gibco-BRL).

in an incubator (25° ± 2 °C, 60 ± 10% RH, 14:10 (L:D) h photoperiod) to daily follow the development of the ectoparasitoid.

The duration and viability of the egg, larval, pupal, and adult stages, the percentage of pupae with no cocoon, the adult longevity, fecundity, and parasitic capacity of the females were determined for each diet. Five replications were conducted for each diet treatment, and all parameters were evaluated simultaneously for parasitoids reared on the natural host (*A. kuehniella*) for comparison purposes.

The performance of each diet was evaluated through cluster (Curi, 1982) and principal components analyses (Silveira Neto, 1986).

To test the capacity of *B. hebetor* individuals to parasitize when reared on different diets and its natural host, 40 terminal-larval stage *A. kuehniella* were placed in plastic cages (10 cm × 10 cm × 3 cm) and a recently emerged (0–24 h) pair of *B. hebetor* adults was introduced and held for 72 h. Treatments were replicated 10 times. This number was based on work by Ullyett (1945). After this exposure time, the adults were removed and the number of paralyzed and parasitized larvae counted. The number of adults and the sex ratio were determined after larval and pupal development of the ectoparasitoids.

To determine the longevity and the ovipositional rate of the ectoparasitoid, recently emerged adult *B. hebetor* reared on the various diets and on the natural host were separated into pairs, placed in glass vials (1 cm × 4 cm) and provided one terminal instar larva of *A. kuehniella* daily until the female died. The longevity of males and females, the number of eggs laid on the host, the total viability of the progeny (percentage of adults compared to the number of eggs laid/female/day), and the sex ratio of the F1 generation were evaluated.

For the statistical analysis, data were compared by the Tukey test, at a probability level of 1 or 5%, with the means transformed by arcsin $\sqrt{(x+\alpha)}/100$.

3. Results and discussion

3.1. Evaluation of artificial diets for rearing B. hebetor

The pre-imaginal development of *B. hebetor* during the different phases of development was influenced by the diet used. Generally, the diets including *A. kuehniella* pupal holotissue (PHT) (diets 4, 5, and 6) required longer developmental periods, approximately 17 days for the whole cycle (egg–adult), which were significantly longer than required with the natural host, *A. kuehniella* (15.5 days) (Table 2). Intermediate values were found for diets 7 and 3 (both contained pupal holotissue of *D. saccharalis* and egg yolk). These were not significantly different from the results with the natural host. Diets 1 and 2, which contained *D. saccharalis* pupal holotissue in their formulation at proportions of 60 and 50%, respectively, required parasitoid developmental periods similar to *A. kuehniella* (Table 2). Guerra (1992), who used the hemolymph of various insect species as the main dietary component, also found differences in the developmental time of *C. grandis* and *B. mellitor* reared in vitro versus on the natural host. The developmental time varied according to the host species, ranging from 11 to 12.5 days.

There was no significant variation in the egg incubation time of *B. hebetor* among the different treatments (about 1.5 days). The larval duration was most affected by the diets. The duration of this phase was always greater on the different diets than on the natural host. On diets 2, 4, 5, 6, and 7, the larval phase was significantly longer (>7 days) than on the natural host (5.8 days). Diets 1 (60% *D. saccharalis* PHT) and 3 (50% plus yeast extract) were not significantly different from the natural host in their effect on larval phase duration.

In contrast, the duration of the pupal phase was little influenced by the diets. This phase was slightly longer on the natural host, though only in comparison with diet 2 (PHT of *D. saccharalis*) (Table 2).

Table 2
Duration of the phases and the total developmental time (egg–adult) of *Bracon hebetor* reared on seven artificial diets and on *A. kuehniella*, the natural host

Treatments	Duration of the phases (days)			
	Egg	Larva	Pupa	Egg – Adult
Diet 1 (*Ds*)	1.58 ± 0.121a	6.27 ± 0.231bc	7.46 ± 0.206ab	15.30 ± 0.200c
Diet 2 (*Ds*)	1.30 ± 0.122a	7.11 ± 0.323ab	7.04 ± 0.088b	15.45 ± 0.244c
Diet 3 (*Ds*)	1.61 ± 0.149a	6.81 ± 0.165abc	7.88 ± 0.220ab	16.30 ± 0.371abc
Diet 4 (*Ak*)	1.79 ± 0.096a	7.56 ± 0.208a	7.65 ± 0.242ab	17.00 ± 0.129a
Diet 5 (*Ak*)	1.75 ± 0.147a	7.62 ± 0.197a	7.65 ± 0.187ab	17.02 ± 0.327a
Diet 6 (*Ak*)	1.76 ± 0.080a	7.43 ± 0.137a	8.05 ± 0.354ab	17.24 ± 0.261a
Diet 7 (*Ds*)	1.64 ± 0.107a	7.41 ± 0.161a	7.60 ± 0.235ab	16.66 ± 0.209ab
A. kuehniella	1.58 ± 0.133a	5.84 ± 0.350c	8.18 ± 0.138a	15.59 ± 0.221bc

Temperature, 25° ± 2 °C; 60% ± 10% relative humidity; and 14-h photophase. Means followed by the same letter in the column are not significantly different—Tukey test, $\alpha = 5\%$. (*Ds* or *Ak*—indicate diets made of pupal holotissues of *D. saccharalis* and *A. kuehniella*, respectively).

Based on these data, it can be seen that the diets made with *D. saccharalis* pupal holotissue at various concentrations (diets 1, 2, 3, and 7) required a developmental period (egg–adult) similar to that on the natural host (*A. kuehniella*). However, among these diets, diets 1 and 3 stood out, because there was no significant difference in any of the developmental duration parameters, when compared to the parasitoids reared on *A. kuehniella*. Pupal holotissue or hemolymph of *D. saccharalis* has also given good results for in vitro rearing of *Trichogramma* species (Cônsoli and Parra, 1996, 1997b).

All of the diets had nutritional qualities that allowed complete development of the parasitoid, to a greater or a lesser extent, indicating that there is a potential for rearing this insect on artificial media and that the system used here was adequate, allowing the larvae to acquire food and develop to the adult stage.

Hatchability was greater on the natural host (88%), similar to the values observed for diets 1, 2, 3, 4, and 7 (Table 3).

Larval viability differed significantly among diets. All of the diets produced viabilities inferior to that of the natural host (92%) (Table 3). The greatest viabilities were found on artificial media 7 and 1, with 59 and 50% larval viability, respectively. These values were higher than, but not significantly different from those obtained for diets 3, 4, and 5, which were about 30%. Although no statistical difference occurred among these values, diets 7 and 1 indicate higher nutritional adequacy for the development of this ectoparasitoid.

Pupal viability was high for all the treatments, with no significant difference among them. However, the viability for the whole developmental cycle varied with diet. The natural host clearly provided the greatest viability (74%). Among the diets, number 7 gave the highest viability (43%) though it was not significantly different from that obtained with diets 1, 3, and 4, which gave 36, 27, and 24% viability, respectively. Diets 2, 5 and 6 gave significantly lower viability than diet 7, but were similar to diet 1, with values below 20%.

This technique requires adaptations and improvements to achieve a useful rearing system, especially the transfer of eggs to the host, because egg inviability, proven to be a consequence of handling, was a major factor affecting total viability.

Another parameter indicative of the nutritional adequacy of artificial diets for rearing *B. hebetor* was the formation of a protective cocoon. Only 2% of the pupae that developed on the natural host lacked a cocoon (Fig. 1). This value was considerably higher for individuals reared on the artificial diets. On diets 1 and 3, 20 and 30% of the pupae, respectively, lacked a cocoon, which was significantly higher than the rate on the natural host. Again, diet 1 was found to be superior, because besides requiring a developmental period similar to that found on the natural host (Table 2) as well as high viability (Table 3), it led to the development of a larger percentage of pupae with cocoons (80%) (Fig. 1), and in this respect it was most similar among the diets to the natural host.

The lack of a protective cocoon in parasitoids reared on artificial diets was also reported by Guerra (1992) for *B. mellitor*. He found that about 65% of the pupae did not produce a cocoon. However, this abnormal type of pupation did not affect pupal survival or adult emergence.

The paralyzing capacity was similar for practically all of the treatments, except for insects reared on diet 3. The *B. hebetor* reared on this diet parasitized less than 50% of the hosts. The other diets gave percentages of paralyzation similar to those observed for parasitoids reared on the natural host (75%), while parasitoids reared on diet 7 were numerically most similar to the value obtained with *A. kuehniella* (Table 4).

Diet 1 stood out because it produced parasitoids with biological parameters similar to those obtained on the natural host as well as a high paralyzation capacity. It also produced individuals with a high viability and a low proportion of pupae lacking cocoons (Table 4). The only drawback was a low percentage parasitism by

Table 3
Viability of the duration of the developmental phases and the total development time (egg–adult) of *Bracon hebetor* reared on seven artificial diets and on *A. kuehniella*, the natural host

Treatments	Viability (%)			
	Egg	Larva	Pupa	Egg–adult
Diet 1 (*Ds*)	73.00 ± 4.64ab	50.63 ± 8.81bc	100.00 ± 0.00a	36.00 ± 6.00bc
Diet 2 (*Ds*)	66.00 ± 5,34ab	26.69 ± 5.46c	100.00 ± 0.00a	17.00 ± 3.39cd
Diet 3 (*Ds*)	75.20 ± 7,48ab	36.03 ± 5.03bc	100.00 ± 0.00a	27.00 ± 4.64bcd
Diet 4 (*Ak*)	78.00 ± 4.06ab	33.89 ± 4.61bc	91.66 ± 5.27a	24.42 ± 3.74bcd
Diet 5 (*Ak*)	56.00 ± 8.57b	34.21 ± 1.62bc	95.00 ± 5.00a	18.25 ± 3.25cd
Diet 6 (*Ak*)	64.00 ± 4.85b	23.53 ± 6.84c	96.67 ± 3.33a	13.17 ± 2.15cd
Dieta 7 (*Ds*)	76.00 ± 6.96ab	59.13 ± 7.35b	98.57 ± 1.43a	43.07 ± 4.70b
A. kuehniella	88.00 ± 5.83a	92.25 ± 4.75a	91.25 ± 3.91a	74.01 ± 6.89a

Temperature, 25° ± 2 °C; relative humidity, 60% ± 10%; and 14-h photophase. Means followed by the same letter in the column are not significantly different—Tukey test, $\alpha = 5\%$. (*Ds* or *Ak*—indicate diets made from pupal holotissues of *D. saccharalis* and *A. kuehniella*, respectively).

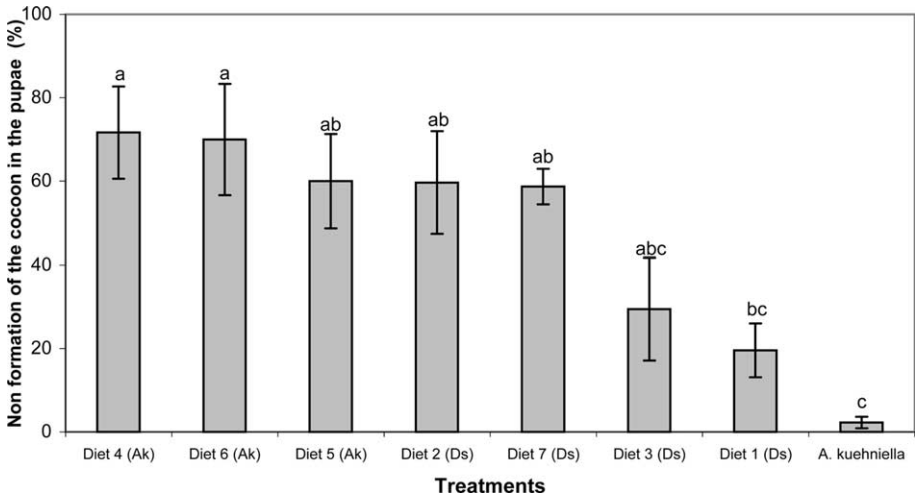

Fig. 1. Mean percentage of pupae *Bracon hebetor* that did not produce a cocoon when reared on the various artificial diets and on *A. kuehniella* (host). The same letters in the columns indicate that there was no significant difference at a 1% probability level by the Tukey test. Means transformed into arcsin $\sqrt{(x+\alpha)}/100$. (*Ds* or *Ak*—indicate diets made of pupal holotissues of *D. saccharalis* and *A. kuehniella*, respectively).

Table 4
Paralyzation and parasitism capacity, during 72 h, of *B. hebetor* reared on seven artificial diets and on the natural host (*A. kuehniella*)

Treatments	Percentage (mean)	
	Paralyzation	Parasitism[a]
A. kuehniella	75.10 ± 3.6a	22.86 ± 2.9a
Diet 7 (*Ds*)	71.49 ± 8.6ab	13.28 ± 3.2ab
Diet 6 (*Ak*)	67.13 ± 6.1ab	12.11 ± 1.7ab
Diet 5 (*Ak*)	66.13 ± 3.8ab	9.86 ± 1.0b
Diet 1 (*Ds*)	60.72 ± 4.4ab	5.8 ± 8.9bc
Diet 4 (*Ak*)	55.81 ± 4.3ab	5.16 ± 1.3bc
Diet 2 (*Ds*)	54.46 ± 6.2ab	3.89 ± 1.3c
Diet 3 (*Ds*)	48.68 ± 8.7b	11.18 ± 1.7ab

Temperature, 25° ± 2 °C; relative humidity, 60% ± 10%; and 14-h photophase. Means followed by the same letter in the column are not significantly different—Tukey test, $\alpha = 5\%$. (*Ds* or *Ak*—indicate diets made of pupal holotissues of *D. saccharalis* and *A. kuehniella*, respectively).

[a] Percentage parasitized compared to the total number of larvae offered.

reared females. Diets 6 and 7 enabled high rates of paralyzation and parasitism, which were comparable to those found for diet 1 and *A. kuehniella*.

Bracon hebetor females inserted their ovipositor into host larvae to paralyze them, thereby facilitating oviposition. This behavior also enables the parasitoid to feed on the hemolymph exuded from the larval body (Beard, 1972; Morrill, 1942). Doutt (1959) reported that before these females initiate oviposition, they try to paralyze all hosts in the vicinity. The number of larvae parasitized varies with temperature and host species (Soliman, 1940). Apparently, artificial diets did not affect this biological behavior.

The mean sex ratio observed for wasps reared on diets 1 and 7 and on the natural host was 0.61. Only the value obtained for diet 3 differed significantly from the expected value ($\chi^2 = 0.54$, df = 1, $p = 0.05$).

Mean longevity of male *B. hebetor* was 8 days on the natural host (*A. kuehniella*). This was significantly longer than that obtained with the various artificial diets (4–6 days) ($F = 11.69$, df = 7, $p = 0.05$). However, female parasitoids reared on artificial diets lived just as long as those reared on the natural host (about 39 days) ($F = 1.51$, df = 7, $p = 0.05$). This is similar to the values reported by Nikam and Pawar (1993).

Bracon hebetor oviposition was continued throughout the females life span. They produced a mean ranging 408 to 576 eggs, both on the natural host and artificial diets. This is considerably more than the mean of 259 eggs per female reported by Nikam and Pawar (1993).

Normal values of female longevity, parasitism, and oviposition rate as well as the total number of eggs produced per female with these diets show the potential of in vitro rearing of this ectoparasitoid. Also, the failure of individuals reared on artificial diets to produce a cocoon did not affect the performance of the first generation adults. However, a nutritional deficiency is likely to appear in succeeding generations reared on the artificial diet because cocoon silk is composed of protein. Lack of a cocoon may indicate a nutritional problem (Craig, 1997).

It was also apparent that the progeny were equally viable in all treatments, with about 80% viability during the developmental stages (egg–adult). However, despite the large quantity of eggs produced by females reared on *A. kuehniella* that had been reared on the different diets and the high viability of the F1 generation, after about the 30th day of adult life of the maternal generation (both those reared on diets and on the natural host), the

sex ratio of the F1 progeny was verified to drop to zero, indicating that unfertilized eggs had been laid, which gave rise to males only. Clearly the females successfully mated because the first eggs laid produced both males and females. Possibly the females depleted the supply of sperm because the males died first. Thereafter, the females produced haploid eggs, giving rise to males only, because this insect has arrhenotokous parthenogenesis.

This increase in male production as the females matured, was also observed by Corrêa-Ferreira and Zamataro (1989) in the egg parasitoid *Trissolcus basalis* (Wollaston). In the genus *Bracon*, unfertilized eggs develop into haploid mates, but fertilized (diploid) eggs produce both males and females (Holloway et al., 1999). Additionally, Ode et al. (1997) showed that female *B. hebetor* from which sperm had been removed only produced males.

Diets 4 and 5 produced similar results according to cluster analysis, with 72% similarity between them. Diet 1 scored 65% in comparison with the natural host. The other treatments had varying degrees of association (Fig. 2).

Diet 1, which also had the best results for in vitro rearing of *Trichogramma pretiosum* Riley and *T. galloi* Zucchi (Cônsoli and Parra, 1997b), provided conditions closest to those of the natural host for rearing *B. hebetor*. Diets 4 and 5 produced quite similar results, however, they appeared to be less appropriate for rearing these ectoparasitoids, due to the low viability and the high percentages of pupae without cocoons. Diet 6 produced similar results to these two diets, demonstrating that the pupal holotissues of *A. kuehniella* were less appropriate for the development of *B. hebetor* than 60% *D. saccharalis* holotissues (diet 1).

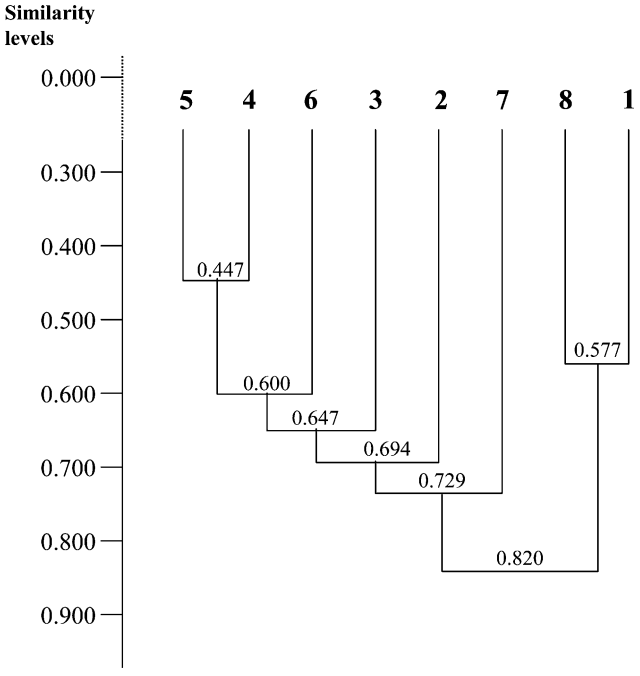

Fig. 2. Phenogram obtained based on the cluster analysis of the seven diets and the natural host tested for rearing *Bracon hebetor*. UPGMA phenogram by mean Euclidian distance. Degree of phenetic correlation (CCC): 0.847; Degree of concatenation (C): 0.667; $P(35\%) = 0.577$ (numbers from 1 to 7 correspond to the diets; 8 corresponds to the natural host, *A. kuehniella*).

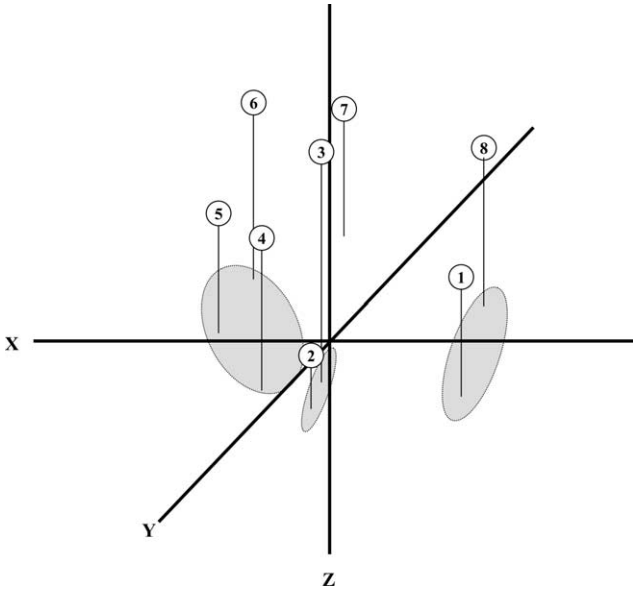

Fig. 3. Three-dimensional distribution of the diets and the natural host by principal components analysis, for the evaluation of the performance of the different diets for the development of *Bracon hebetor* (numbers 1–7 correspond to the diets; 8 corresponds to the natural host, *A. kuehniella*).

Larval viability, developmental cycle duration, and the capacity to paralyze were the characters most responsible for the grouping of the different treatments (Principal Components Analysis, with over 70% significance) (Fig. 3).

Even though diet 7 had high rates of viability (statistically similar to diet 1), it differed from the natural host/diet 1 group because the wasps reared on diet 7 had a delayed developmental cycle (Fig. 3).

Therefore, we conclude that diet 1 is the most adequate for rearing *B. hebetor*, based on the biological parameters of development time, larval and pupal viability, and female longevity, fecundity, and aggressiveness.

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