Development of the Parasitoid *Chelonus insularis* (Hymenoptera: Braconidae) in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Larvae Reared on Castor Bean and Maize Leaves[1]

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Abstract The use of artificial diets for rearing natural enemies is an expensive technique with negative implications in the development of parasitoids. The aim of this study was to determine the effects on the development of the parasitoid *Chelonus insularis* Cresson (Hymenoptera: Braconidae) using as the host *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae reared on castor bean (*Ricinus communis* L.) and maize (*Zea mays* L.) leaves. Twenty-five egg masses of *S. frugiperda* were exposed to adult parasitoids of *C. insularis*. One hundred twenty-three larvae were fed with castor bean leaves and 309 larvae with maize. Survival of *S. frugiperda* larvae and emergence of healthy adults of *C. insularis* were recorded. Durations of the developmental stages, weight per parasitoid, length of the radial cell, and total length of the forewing also were recorded. No significant differences were determined between the two host plants with respect to survival of *S. frugiperda* larvae or the emergence of healthy adults of *C. insularis*. Durations of the developmental stages of the parasitoid were longer on castor bean leaves than on maize. The length of the radial cell and the total length of the forewing were greater on parasitoids that emerged from castor bean-reared larvae than those reared on maize for both females and males. The weight per parasitoid did not differ. *Chelonus insularis* can be maintained in *S. frugiperda* larvae reared on castor and maize leaves but, based on these results, the use of castor bean leaves favors the size of the parasitoid.

Key Words biological control, fall armyworm, *Ricinus communis*, *Zea mays*

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is a polyphagous insect, and in the Americas, it has approximately 353 hosts belonging to 76 botanical families, mainly Poaceae (Montezano et al. 2018). It is one of the main pests on maize crops (*Zea mays* L.) (Poales: Poaceae), causing losses of 8–60% of the expected yield (Andrews 1980). The control of this insect is primarily by application of synthetic insecticides. In Mexico, the annual applications total about 3,000 t of active ingredient (Blanco et al. 2014). It is necessary to find management strategies that reduce or avoid the excessive use of these chemical insecticides, such as the use of natural enemies, particularly parasitoids. In Mexico,

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approximately 87 species of parasitoids, mainly hymenopterans and dipterans, have been recorded from *S. frugiperda* (Bahena-Juárez and Cortez-Mondaca 2015). *Chelonus insularis* Cresson (Hymenoptera: Braconidae) is an egg-larval endoparasitoid, koinobiont, of *S. frugiperda* with records of natural parasitism rates between 1.0 and 60.3%, and it is present in at least 27 of the 32 states of the Mexican Republic (González-Maldonado et al. 2014). For these reasons *C. insularis* is considered one of the main biological control agents of this pest with a great potential for use in biological control by augmentation (Bahena-Juárez and Cortez-Mondaca 2015).

Rearing and reproduction of a parasitoid under laboratory conditions involve the rearing of an insect host and a host plant or artificial diet on which the insect host feeds. The use of artificial diets for rearing natural enemies is an expensive technique with negative implications in the development of parasitoids (Cônsoli and Grenier 2010, van Lenteren and Bigler 2010). In addition, it is not recommended to use artificial diets for the rearing of endoparasitoids (Greany et al. 1984). It would be advisable to rear *C. insularis* parasitoids on their natural host (*S. frugiperda*), which, in turn, are reared on plants that are easy to cultivate. Recently, castor bean (*Ricinus communis* L.) (Malpighiales: Euphorbiaceae) leaves have become widely used for rearing of *Spodoptera* spp. larvae (Alfazairy et al. 2012, Bayu and Krisnawati 2016, Martínez-Martínez et al. 2015), primarily because the larvae and pupae weigh more than those reared on other host plants (Banerjee and Ray 1995, Bayu and Krisnawati 2016, Cabezas et al. 2013). However, some studies have reported the presence of secondary metabolites in the plant, such as ricin oil, ricinin, and trypsin inhibitors, which have antagonistic effects on larval development (Ramos-López et al. 2010, Rossi et al. 2012, Saeed et al. 2017).

Martínez-Martínez et al. (2015) compared the development of *S. frugiperda* reared on castor bean and maize leaves, without determining statistically significant differences between the evaluated variables, except for the duration of pupa stage, which was longer on castor bean leaves. In a similar experiment, Bahena-Juárez and Zamora-Equihua (2012) reported a prolonged duration of the developmental larval stage and greater weight and size of pupae when *S. frugiperda* was reared on castor bean leaves compared with maize leaves and an artificial diet. It is possible to rear *S. frugiperda* larvae on castor bean leaves, but the effect of diet on the development of natural enemies is unknown. In some studies, it has been reported that the secondary metabolites of plants do not affect the development of the insect host but do affect the development of endoparasitoids (Barbosa et al. 1991, Gols et al. 2008, Kauffman and Kennedy 1989, Sarfraz et al. 2008, Sétamou et al. 2005). Therefore, the aim of this study was to determine the effects on the development of the parasitoid *C. insularis* using as the host *S. frugiperda* larvae reared on castor bean and maize leaves.

Materials and Methods

The study was conducted at the Biological Control laboratory of the Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Unidad Oaxaca, located in Santa Cruz Xoxocotlán, Oaxaca, Mexico, under ambient environmental conditions. *Spodoptera frugiperda* egg masses used in the study were obtained from a laboratory colony maintained on maize leaves. For the study, 25 freshly oviposited egg masses were collected from the colony and simultaneously exposed for parasitization by placing one to two masses in individual 1-L capacity plastic bottles with 8–11 *C. insularis* adults for approximately 7 h. The adult parasitoids were obtained by collecting parasitized *S. frugiperda* larvae infesting maize fields at Oaxaca, Mexico.

As per the methods of Martínez-Martínez et al. (2015), the exposed egg masses were then divided in half, and each half was placed individually in plastic Petri dishes (100 × 15 mm) on fresh leaves to ensure that the enclosed neonates had available food. Half of each egg mass was reared on maize leaves obtained from a crop without agrochemical applications, and the other half of the egg mass was reared on castor bean leaves obtained from plants at Santa Cruz Xoxocotlán, Oaxaca, Mexico. Twenty of second- or third-stage (e.g., presence of the cephalic capsule from exuviae) larvae were randomly selected by host plant type and transferred individually into plastic Petri dishes (60 × 15 mm) containing the appropriate food to further monitor survivorship and development. Castor bean leaves were fed to 123 *S. frugiperda* larvae that hatched from 15 egg masses; of those, 62 larvae were individually placed in Petri dishes as second instars and 61 as third instars. In the same manner, 309 larvae from 22 egg masses were fed on maize leaves and, of those, 263 second instars and 46 third instars were individually placed on maize in small Petri dishes. Egg or larvae that were exposed to *C. insularis* but not used in further observations had either dehydrated (eggs) before hatching or had died during molting (larvae).

Spodoptera frugiperda larvae were supplied with fresh foliage daily, *ad libitum*, until the emergence of the parasitoid larvae or until the *S. frugiperda* larvae began pupating. The number of parasitized and nonparasitized *S. frugiperda* larvae was recorded, and the percentage of *C. insularis* parasitism on *S. frugiperda* eggs was calculated from the total number of emerged adults (moths and parasitoids). The number of healthy adult parasitoids that emerged by host plant type and the durations of the developmental stages of *C. insularis* (e.g., larva, prepupa, pupa, hatching to adult emergence) were recorded and grouped according to the instar in which *C. insularis* larvae emerged from the host.

When *C. insularis* adults emerged, they were confined in 6-L capacity plastic bottles covered with organza fabric to allow air exchange. Inside the plastic bottle, a paper strip was placed with few drops of honey and a piece of cotton wetted with water for food. When more than 20 parasitoids were collected, the bottle was placed in the freezer for 30 min to kill them, and then they were grouped by sex. The proportion of females, by host plant type, was determined. The parasitoids were weighed on an analytical balance (Sartorius LP620P; Sartorius AG, Göttingen, Germany), and the weight (mg) per parasitoid was determined from the total number of weighed individuals. The length of the radial cell and the total length of the wing (distance from tegula to wingtip) of a forewing of each parasitoid were measured (Owen 2012) using a stereoscope microscope (Carl Zeiss Stemi DV4; Zeiss Group, Göttingen, Germany) equipped with a lens caliper.

Statistical analysis. All data were analyzed using the independent-samples *t* test (variables with normal distribution) and the Mann–Whitney *U* test (variables with different distribution than normal) (McKnight and Najab 2010) for statistical analysis, with a confidence level of 95%. The Kolmogorov–Smirnov and Shapiro–

Wilk tests were used to determine the normality of the variables studied. All statistical analyses were performed using the SPSS-Windows 25.0 program (SPSS, Chicago, IL). Differences were analyzed by host plant type and were considered significant when $P \leq 0.05$. Data are presented as mean ± standard error of the mean (SEM).

Results and Discussion

The number of individual *S. frugiperda* larvae reared on castor bean leaves compared with maize leaves may be related to the change in the diet or the physiologic deterioration caused by the development of the parasitoid when it is feeding inside the host. Also, the deleterious effect in the early development of *S. frugiperda* larvae in the feeding with castor bean leaves may be due to the presence of secondary metabolites (Ramos-López et al. 2010, Rossi et al. 2012, Saeed et al. 2017). Similar data were reported by Martínez-Martínez and Arzuffi-Barrera (2008) when they incorporated huamuchil (*Pithecellobium dulce* [Roxb.] Benth) powders into the host diet.

Survival of *S. frugiperda* larvae. During the course of the experiment, the survival percentage (±SEM) of *S. frugiperda* larvae was 78.9 ± 7.1% when reared on castor bean leaves and 71.0 ± 4.1% on maize leaves, without statistically significant differences between the median number of live larvae and host plant types (Mann–Whitney *U* test, $U = 127.00$; $P = 0.24$). Martínez-Martínez et al. (2015) determined a survival percentage of *S. frugiperda* nonparasitized larvae higher than 85.6% when larvae were reared on maize and castor bean leaves. This suggests that our results were due to the effect of parasitism because the parasitoid feeds and develops inside its host resulting in physiological damages (Martínez-Martínez and Arzuffi-Barrera 2008). Data show that *S. frugiperda* feeding with castor bean leaves do not affect the larvae development, previously exposed to parasitism, after the third instar. *Spodoptera frugiperda* larvae may be adapted to the feeding with castor bean leaves because it is a host plant of this insect (Montezano et al. 2018), and in Mexico, it is part of the ruderal vegetation (Espinosa-García and Villaseñor 2017, Rangel-Landa et al. 2017), and the castor bean plants growing around the maize crops may be one of the main alternate hosts of the *S. frugiperda* larvae. Several studies have demonstrated the adaptation of *Spodoptera* spp. larvae to castor bean leaves feeding (Banerjee and Ray 1995, Cabezas et al. 2013, Bayu and Krisnawati 2016). This suggests the possibility of developing, under laboratory conditions, a population of *S. frugiperda* well adapted to feeding on castor bean leaves, but the behavior of the *C. insularis* parasitoid needs to be evaluated. The percentage of *C. insularis* parasitism on *S. frugiperda* eggs was 81.4%.

Emergence and duration of the developmental stages of *C. insularis*. The emergence percentage of healthy *C. insularis* adults reared on castor bean leaves was 96.0 ± 3.6% and 82.2 ± 5.1% on maize leaves, without statistically significant differences between the median number of healthy adult parasitoids emerged and host plant types (Mann–Whitney *U* test, $U = 130.00$; $P = 0.28$). In addition, some problems were observed during the emergence of *C. insularis* adults from larvae, mainly those reared on maize leaves. The deformed adults were parasitoids that completed their pupal stage outside the cocoon. Some other adult parasitoids were trapped inside the leaves when the food was dehydrated, or a part of the body remained trapped in the cocoon (Table 1).

Our results with the emergence of healthy *C. insularis* adults are opposed to those reported by Bahena-Juárez and Zamora-Equihua (2012) with the parasitoid *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) reared on *S. frugiperda* larvae. They recorded 84% of emergence of healthy adults reared on castor bean leaves and 94% on maize leaves. Therefore, the same host plant types on *S. frugiperda* may have different effects on the survival of parasitoids, depending on the species. It is also possible that *C. insularis* has a higher tolerance than *C. sonorensis* to the secondary metabolites present in castor bean leaves, because *C. insularis* remains within the host for a longer period than *C. sonorensis*.

Thompson and Redak (2005) found that the *Manduca sexta* L. (Lepidoptera: Sphingidae) larvae parasitized by *Cotesia congregata* Say (Hymenoptera: Braconidae) prefer diets with a high lipid content, apparently because the fatty acid reserve in the host favors the emergence of the parasitoids (Murugan et al. 2000). Therefore, it would be appropriate to determine the chemical profile of the castor bean leaves used in the feeding of *S. frugiperda* larvae in our study. Meanwhile, the results of the research conducted by Hatem et al. (2016) show that the age of the female that parasitizes the larval host is linked to mortality of the pupae and thus directly impacts the percentage of adult emergence. These aspects should be considered for future evaluations to optimize the rearing of *C. insularis*.

The period of time from parasitism to eclosion of *S. frugiperda* eggs was 3.5 d after the exposure to *C. insularis* adults. The emergence of parasitoid larvae from the host varied with the host plant type. When host larvae were fed on maize, parasitoid emergence started during the second and continued to the sixth instar of *S. frugiperda*, whereas on castor bean, emergence occurred from the third to sixth instar. On maize leaves, the greatest number of parasitoid larvae emerging from the host were in the fourth instar, whereas on castor bean leaves, a similar number was recorded for the fourth and fifth instar. Regarding the duration of the developmental of *C. insularis*, significant differences were found between the host plant types and all the developmental stages of parasitoids that emerged from the host during the fourth instar. For *C. insularis* larvae that emerged from the host during the fifth instar, significant differences were found between host plant types, the duration of the pupal stage, and from hatching to adult emergence. The durations of the developmental of *C. insularis* was longer when the host was reared on castor bean than on maize leaves. The duration from hatching to adult emergence was shorter when parasitoid larvae emerged from the host during the fourth instar, followed by parasitoid larvae that emerged from the host during the third instar; this duration was longer when parasitoid larvae emerged from hosts that molted more than four times regardless of host plant types (Table 2).

The variation recorded for the instars in which *C. insularis* larvae emerged from the host may have resulted from a lack of controlled temperature, relative humidity, and photoperiod, as indicated by Barraclough et al. (2014). It is common under such conditions that an extra number of instars is associated with the production of smaller insects; however, for hosts and parasitoid insects, the extra instars compensate for the low growth rates of larvae before they reach the pupa stage (Barraclough et al. 2014). In the experiment conducted by Rezende et al. (1994), all *C. insularis* larvae emerged from the host during their fifth instar when *S. frugiperda*

Table 1. Mean percentage emergence (±SEM) of healthy *C. insularis* adults and problems observed in *S. frugiperda* larvae reared on castor bean and maize leaves.

Host Plant Types	Emergence of Healthy Adults	Deformed Adults	Trapped	
			Inside Leaves	In Cocoon
Castor bean	96.05 ± 3.56 (78)	0.00	3.57 ± 3.5 (1)	0.38 ± 0.38 (1)
Maize	82.20 ± 5.12 (152)	8.41 ± 2.90 (11)	9.39 ± 4.95 (10)	0.00

Numbers in parentheses indicate the number of parasitoids that represent the percentage indicated.

Table 2. Duration (days ± SEM) of developmental stages of *C. insularis* in *S. frugiperda* larvae reared on castor bean and maize leaves, according to the instar in which *C. insularis* larvae emerged from the host.

	Developmental Stages				
	Larva	**Prepupa**	**Pupa**	**Hatching to Adult Emergence**	***n***
Second instar					
Castor bean	—	—	—	—	0
Maize	6.50 ± 0.61	8.62 ± 1.09	8.00 ± 0.41	23.12 ± 0.83	4
Third instar					
Castor bean	9.60 ± 0.73 $a^{\ddagger}$	7.80 ± 1.39 $a^{\ddagger}$	9.20 ± 0.20 $a^{\ddagger}$	26.60 ± 0.99 $a^{\dagger}$	5
Maize	9.94 ± 0.28 a	5.97 ± 0.31 a	8.97 ± 0.19 a	25.00 ± 0.39 a	32
Fourth instar					
Castor bean	11.47 ± 0.48 $a^{\ddagger**}$	4.78 ± 0.15 $b^{\ddagger**}$	10.19 ± 0.20 $a^{\ddagger**}$	26.32 ± 0.53 $a^{\ddagger**}$	32
Maize	9.57 ± 0.17 b	5.79 ± 0.14 a	8.88 ± 0.21 b	24.24 ± 0.24 b	91
Fifth instar					
Castor bean	12.06 ± 0.33 $a^{\ddagger}$	5.60 ± 0.23 $a^{\ddagger}$	10.06 ± 0.25 $a^{\ddagger*}$	27.71 ± 0.39 $a^{\ddagger*}$	35
Maize	11.95 ± 0.30 a	5.29 ± 0.27 a	9.10 ± 0.33 b	26.40 ± 0.41 b	39
Sixth instar					
Castor bean	14.12 ± 0.61 $a^{\dagger}$	4.75 ± 0.49 $a^{\dagger}$	10.12 ± 0.44 $a^{\dagger}$	29.00 ± 0.57 $a^{\dagger}$	8
Maize	14.79 ± 0.52 a	5.71 ± 0.57 a	9.00 ± 0.76 a	29.50 ± 0.69 a	7

n, number of individuals that constitute the sample size. Values with the same letter within the same instar do not differ significantly ($P > 0.05$): $^{\dagger}t$ test, ‡Mann–Whitney *U* test.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

Table 3. Mean weight per parasitoid (mg ± SEM), length of radial cell, and total length of forewing (mm ± SEM) of *C. insularis* adults in *S. frugiperda* larvae reared on castor bean and maize leaves.

	Weight per Parasitoid (mg)		Radial Cell (mm)
Host Plant Types	♀	♂	♀
Castor bean	4.97 ± 0.34 a† (3)	3.86 ± 0.43 a‡ (3)	0.93 ± 0.02 a$^{\ddagger *}$ (18)
Maize	4.23 ± 0.02 a (2)	3.25 ± 0.15 a (3)	0.88 ± 0.01 b (50)

Numbers in parentheses indicate the sample size. Values with the same letter within the same column do not differ significantly ($P > 0.05$): † *t* test, ‡ Mann–Whitney *U* test.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

was reared on maize leaves. In this study, when *S. frugiperda* was reared on maize leaves, the greatest number of *C. insularis* larvae emerged from the host during the fourth instar (91 larvae). For those reared on castor bean leaves, the number was similar between the fourth and fifth instar of *S. frugiperda* (32 and 35 larvae, respectively; Table 2). This is directly related to the instar in which the *S. frugiperda* larvae were transferred to the individual Petri dishes with 263 second instars transferred to maize and 62 second instars and 61 third instars transferred to castor bean.

Our results suggest that the feeding of *S. frugiperda* larvae with castor bean leaves prolongs their developmental period and, in turn, extends the time for *C. insularis* larvae to reach the adult stage (Table 2). Similar data were reported by Martínez-Martínez and Arzuffi-Barrera (2008) when adding huamuchil powders to the larval diet. Bahena-Juárez and Zamora-Equihua (2012) reported for *C. sonorensis* a period from oviposition to adult emergence of 15.1 d on castor bean leaves and 13.1 d on maize. They also indicated that the larval feeding did not influence the duration of the life cycle of parasitoid. Furthermore, the variation between the duration of the developmental stages of *C. insularis* in *S. frugiperda* larvae reared on castor bean and maize leaves may be associated with differences in the nutrient content (e.g., proteins, carbohydrates, lipids, water, and amino acids) or the presence of secondary metabolites of the host plants (Barbosa et al. 1991, Gols et al. 2008, Murugan et al. 2000, Sarfraz et al. 2008).

Weight and wing length of adult parasitoids of *C. insularis*. In this study, the proportion of *C. insularis* female parasitoids emerged from host larvae fed on castor bean leaves was 0.23, whereas that proportion was 0.35 on maize. Further studies of this result are necessary to quantify and address the possibility or improving the proportion of females emerging from parasitized *S. frugiperda*.

No significant differences were revealed between the weight per parasitoid and host plant types for both females and males, possibly because the sample size was small (Table 3). Barraclough et al. (2014) reported that the cocoons of the *Meteorus pulchicornis* (Wesmael) (Hymenoptera: Braconidae) parasitoid were heavier when the parasitoid larva took a longer time to emerged from the host. They indicated that a prolonged developmental period of the host is associated with the production of

Table 3. Extended.

Radial Cell (mm)	Total Length (mm)	
♂	♀	♂
0.91 ± 0.01 a$^{\ddagger **}$ (59)	3.68 ± 0.04 a$^{\dagger **}$ (18)	3.60 ± 0.02 a$^{\ddagger **}$ (59)
0.86 ± 0.01 b (93)	3.52 ± 0.03 b (49)	3.41 ± 0.03 b (93)

larger parasitoids, and our results corroborate their report, as *C. insularis* larvae took a longer time to emerged from the host when *S. frugiperda* was reared on castor bean leaves compared with maize. However, in the research conducted by Martínez-Martínez and Arzuffi-Barrera (2008), the huamuchil powders added to the diet of *S. frugiperda* prolonged the developmental period of *C. insularis*, but the parasitoids weighed less than on the control diet. It is possible that *C. insularis* does not tolerate secondary metabolites present in huamuchil than those found in castor bean.

There were significant differences for host plant types and the length of the radial cell and total length of the forewing in both sexes. In general, female and male *C. insularis* parasitoids reared on hosts with castor bean leaves showed greater weight and wing length than the parasitoids obtained from hosts fed with maize leaves (Table 3).

The use of castor bean leaves may be feasible for rearing *C. insularis* parasitoids in *S. frugiperda* larvae, mainly in areas where the plant is part of the ruderal vegetation or is easily accessible; however, it is necessary to determine the effect of host feeding on parasitoid development over several generations to preserve parasitoid quality parameters. Alfazairy et al. (2012) report that *S. littoralis* Boisduval (Lepidoptera: Noctuidae) larvae can be reared on castor bean leaves for nine generations with more than 70% adult emergence rate. *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae are capable of developing resistance or tolerance to secondary metabolites produced by host plants under selective pressure (Wu et al. 2010), but it is unknown whether parasitoids can respond in the same way as the host. Bahena-Juárez and Zamora-Equihua (2012) point out that there are variations in the developmental period of *C. sonorensis* from the second generation, when *S. frugiperda* was reared on castor bean leaves.

In summary, our results demonstrate that feeding *S. frugiperda* larvae on castor bean leaves prolonged the duration of the developmental stages of *C. insularis* parasitoids compared to those reared on maize leaves but, on castor bean leaves, the weight per parasitoid, the length of the radial cell, and the total length of the forewing, in both male and female parasitoids, were higher. Therefore, *C. insularis* can be maintained in *S. frugiperda* larvae reared on castor bean and maize leaves, but the use of castor bean leaves favors the size of the parasitoid. Finally, it would be useful to evaluate quality control parameters over several generations to determine the feasibility of using castor bean leaves in mass reproduction techniques for *C. insularis* or within integrated pest management (IPM) programs.

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