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## COMADIA REDTENBACHERI (LEPIDOPTERA: COSSIDAE) PUPAL DEVELOPMENT IN THE LABORATORY

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### ABSTRACT

Wild populations of *Comadia redtenbacheri* Hammerschmidt (Lepidoptera: Cossidae), known in its larval stage as the agave redworm, are gathered intensively for sale and consumption as food. To define adequate conditions for their pupation in confinement, the effect of handling larvae during collection, their weight when induced to pupate, substrate type and the moisture content provided during this stage of development, were evaluated over 2 consecutive yr. These factors were analyzed by logistic regression (PROC GENMOD,  $\alpha = 0.05$ ), relative to the emergence of adults. The relationship between larval weight and adult sexual gender was analyzed with a contingency table. The larvae exhibited adaptation to different substrates used for pupation, and substrates could be reused. The heavy clay content soil from areas where agaves grow, mixed with vermiculite (50:50), was the most suitable substrate (estimated value 0.7304). Moisture had no significant effect on pupation. Emergence of adults was significantly greater from larvae that had not been handled roughly as those acquired from commercial vendors. According to the Chi-square test, the capacity to pupate by fifth instar larvae that weighed 0.30 to 0.39 g was not significantly different from that of the sixth and seventh instars that weighed 0.40 g or more. Males emerged mainly from cocoons produced by the smallest larvae, while females emerged mainly from cocoons by heavier larvae.

Key Words: agave redworm, edible insect, insect rearing, *Agave* spp.

### RESUMEN

Las poblaciones silvestres de *Comadia redtenbacheri* Hammerschmidt, (Lepidoptera: Cossidae), conocido en su estado larval como gusano rojo del maguey, son colectadas de manera intensiva para su venta y consumo como alimento. Con el objeto de definir las condiciones adecuadas para la pupación en confinamiento, se evaluaron durante dos años consecutivos el efecto de la manipulación de las larvas durante la colecta, su peso al ser inducidas a pupar, así como el tipo de sustrato y la humedad proporcionados durante esta etapa de desarrollo. Los factores se analizaron mediante regresión logística (PROC GENMOD,  $\alpha = 0.05$ ), con base en la emergencia de adultos. La relación entre el peso larval y el sexo de los adultos se analizó con una tabla de contingencia. Las larvas se adaptaron a los diferentes sustratos utilizados para la pupación, los que incluso pueden ser reutilizados. La mezcla de suelo arcilloso de las áreas magueyeras con vermiculita en proporción 50:50 fue la más adecuada (valor estimado 0.7304). La humedad no tuvo un efecto significativo en el proceso de pupación. Al utilizar larvas sin manipulación con fines comerciales, la emergencia de adultos aumentó significativamente con respecto a las manipuladas. De acuerdo con la prueba de Chi Cuadrada, las larvas de quinto instar con peso de 0.30 a 0.39 g que logran pupar y emerger como adultos, no presentan diferencias significativas con las larvas de sexto y séptimo instares y peso de 0.40 o más. Los machos emergieron principalmente de puparios producidos por las larvas más pequeñas, mientras que las hembras lo hicieron de puparios que albergaron a larvas de mayor peso.

Palabras Clave: gusano rojo de maguey, insectos comestibles, cría de insectos, *Agave* spp.

Fifteen species of the Cossidae are distributed in the USA, but *Comadia redtenbacheri* Hammerschmidt (Lepidoptera: Cossidae), the agave

redworm, is the only cossid species reported in Mexico. It has the following synonyms: *Zeuzera redtenbacheri* Hamm., 1848; *Bombix agavis*

Blásquez, 1870; *Hypopta chilodora* Dyar, 1910 and *H. redtenbacheri* Dyar, 1937 (Brown 1976). The main agave (Aspargales: Asparagaceae) species in whose rhizomes the agave redworm lives are *Agave salmiana* Otto ex Salm, *A. mapisaga* Trel. and *A. atrovirens* Karw. ex Salm (Camacho et al. 2003). Under field conditions, the eggs of *C. redtenbacheri* are deposited in mass by females at the bases of the agave leaves. Eggs have an incubation period of 30 to 35 days. After the eggs hatch, the larvae pass to the rhizome of the plant and metamorphose through 7 instars in approximately eight months (Hernández-Livera et al. 2005; Llanderal-Cázares et al. 2007). Mature larvae leave the agave to pupate in the soil about 5 cm below the surface (Almanza-Valenzuela 2007). The duration of the pupal period is 3.5 to 5.0 months. The adults live only for 3 to 5 days, because they have atrophied mouth parts that prevent feeding. The life cycle is completed in about 1 year (Llanderal-Cázares et al. 2007). Because *C. redtenbacheri* larvae are suitable for human consumption, they are intensively gathered, causing their over exploitation and consequently the decline of the *C. redtenbacheri* population and that of its host, which is unearthed to extract the larvae from the rhizome and which is not replanted. Therefore, new knowledge is needed to assure the sustainable use of this food insect (Llanderal-Cázares et al. 2010; Zetina et al. 2011). Moreover there is interest in rearing *C. redtenbacheri* larvae for commercialization, and in inducing pupation to obtain adults for various uses including basic studies and for their release in production units (Nolasco et al. 2002; Camacho et al. 2003, 2005; Llanderal-Cázares et al. 2007).

The pupa is an important developmental stage from a physiological and morphological perspective. During this stage, development of the major structural differences between immature and adult stages occurs: muscles are formed and inserted into the cuticle and wings and genitalia become fully developed (Mehta 1933; Chapman 1998; Gullan 2010). Many lepidopteran species require one yr to complete their life cycle and have developed an obtect-type pupa, in which a secretion produced by the larvae fastens wings and legs to the body, and, also, the abdomen is relatively immobile (Chapman 1998; Triplehorn & Johnson 2005). To pupate, lepidopteran larvae construct an ovoid cocoon or pupal case, which they consolidate with silk from their salivary glands, soil particles, small stones and even plant fragments (Collin et al. 2010). The cocoon guarantees substantial protection against predators, microorganisms and severe changes in climatic conditions (Guillott 2005).

Initially the pupa of *C. redtenbacheri* is almost white, but with the passing of days, it darkens. At the tip of the pupal abdomen on the ventral side there are cuticular marks, typical of either males

or females that permit the individuals to be sexed (Llanderal-Cázares et al. 2007). The pupa has a series of hooked spikes on the dorsum of the abdomen, which aids in digging up to the soil surface for adult emergence (Brown 1976). Larvae and pupae are affected by microorganisms and by the parasitoids, *Lissonota fascipennis* Townes (Hymenoptera: Ichneumonidae) (Zetina et al. 2009) and *Acantholespesia texana* (Aldrich & Webber) (Diptera: Tachinidae) (Zetina et al. 2012), which affect red maguay worm populations. In programs for the breeding of the insect is important to utilize larvae that are of suitable quality to be reared to adults for mating and oviposition (Zetina et al. 2011).

Duration of pupation in any species is determined by both biotic and abiotic factors. The most important of the latter are RH, which affects dehydration, temperature, which has an influence in the duration of the life cycle, and photoperiod, which regulates daily or seasonal physiological activities (Véjar 2004). Soil is another important factor because its texture and structure can limit insect development when different from that to which a species is adapted (Romero & Villanueva 2000). The combination of environmental factors determines successful pupation, which is a critical stage in the insect's development that can become complicated under confinement and can limit adult emergence (Gillott 2005). Environmental components are easily measured, but their impacts on insect populations are difficult to determine; and if these effects are not controlled, it is impossible to establish a rearing method (Montoya et al. 2008).

Information on the pupal stage of the agave redworm is essential for its management under confined conditions. Which of the instars are able to pupate is still undefined. Currently, to establish colonies of this species for research or other purposes, larvae must be gathered in the field. Thus, this study was conducted to define the conditions adequate for pupation of *C. redtenbacheri*, relative to larval handling during their gathering and collection, weight at the onset of pupation, and the type and moisture content of substrate required for successful pupation.

## MATERIALS AND METHODS

### Description of the Larval Collection Area

Larvae used as breeding stock were obtained from different sites in the northwestern region of the State of Mexico (Ixtapaluca, Coatepec, Pyramids, Otumba, San Pedro, Tezoutlalpan and Tlanguistengo), as well as from nearby communities belonging to the state of Hidalgo (El Puerto, Pozos and Peña Colorada). Diverse sites were selected to guarantee broad genetic variability, an important aspect in insect rearing, according to Parra (1979).

These sites form part of the province of the neovolcanic belt, which is formed by the subprovince of the plains and mountains of Querétaro and Hidalgo and the subprovince of the Anahuac lakes and volcanoes. It forms part of the hydrological regions of the Lerma-Santiago and El Balsas, and a small portion of that of the Pánuco. The climate in this area is temperate subhumid with summer rains and warm subhumid winters (INEGI 2012).

Subprovince of the Plains and Mountains of Querétaro and Hidalgo

This subprovince penetrates the northern part of the state of Mexico at 3 points and covers 1,415.29 km<sup>2</sup> (6.08% of the state's total area). The municipalities included in this area are Polotitlán and Soyaniquilpan, as well as part of Jilotepec, Aculco, Acambay, Chapa de Mota and Hueyoptla. Although there is no great diversity of soils, they are very fertile and suitable for agriculture and livestock raising (INEGI 2012).

Subprovince of the Anáhuac Lakes and Volcanoes in the State of Mexico

This subprovince occupies 14,315.69 km<sup>2</sup> (61.6%) of the state's total area, encompassing 84 municipalities and part of another 18. There are 27 soil types, among them are different classes of cambisols, fluvisols, and solonchaks, as well as eutric planosol, calcareous phaeozem, calcareous vertisol, calcareous regosol and humic gleisol (INEGI 2012).

Four samples were collected, 2 in Sep 2010 and 2 in Oct 2011. The samples collected in both yr were of 2 types: a) "no manipulation" when larvae were extracted from the agave rhizome by a single person and transported carefully to avoid excessive handling, and b) "commercial", which comprised larvae acquired at different places where diverse persons were selling the larvae they had collected without special care during their extraction from the host agave rhizome and, generally, with much manipulation during the process of gathering, collection and sale.

Larvae of sixth and seventh instars according to Hernández-Livera et al. (2005) weighing 0.40 g to almost 1.0 g were selected since, considering previous observations, they probably would initiate pupation once placed in a suitable substrate. Fifth instars, which weighed between 0.30 and 0.39 g, were confined to chambers with soil to define their pupation capacity and the possible relationship between larval weight and adult sex, and for comparison with the last 2 instars weighing 0.40 g or more.

Laboratory Work

In 3 experiments conducted in 2010, the selected larvae were induced to pupate between

Sep 30 and Oct 12. In order to integrate the effects of different conditions evaluated on the population collected in 2010 and to test a more easily managed substrate, a fourth experiment was set up from 10 to 15 Oct 2011. Treatments were kept in 24 h of darkness at 40-50% RH and 20 ± 5 °C by using a humidifier and heaters. Because of their gregarious habit, *C. redtenbacheri* larvae under confined conditions tend to construct common pupation chambers, causing contamination and mortality. It was therefore necessary to keep the individuals separate. The receptacles used as individual pupation chambers were made of polyduct tubes (10 cm long × 3.18 cm diam), placed in 36 × 29 × 12 cm plastic trays, each with a holding capacity for 50 tubes. Into each of the tubes, a layer of substrate 5 cm deep was added for pupation, following Roubos & Liburd (2010) for pupation of the species, *Dasineura oxycoccana* (Johnson) (Diptera: Cecidomyiidae) (Fig. 1). Cocoons from each of the first 3 experiments were destructively sampled to determine the degree to which the individuals had advanced in their transformation into pupae.

#### 1. Evaluation of Substrates and Moisture Content on Pupation and Adult Emergence

The substrate treatments, with and without moisture, consisted of 4 trays each with 50 "commercial" larvae per type of substrate, i.e., S1, soil from the areas of agaves where the larvae were collected; S2, 75:25 mixture of this soil and vermiculite (INSULEX®, Aislantes y acústicos, S. A.), and S3, peat moss (Kekklila®). Two trays of each substrate were moistened with 100 mL water sprayed with an atomizer every 3 wk. In this experiment the numbers of emerged adults were recorded and used as the dependent variable analyzed with logistic regression (PROC GENMOD), to determine the odd-ratio of adult emergence vs. total larvae by soil type and humidity treatment.

The basic structure of the linear model used on the logistic regression structure was:

$$\mu = \beta_0 + \beta_1 I_{CH} + \beta_2 \beta_{S1} + \beta_3 I_{S2}$$

where  $\mu$  is the linear model,  $\beta_0$  is the intercept representing the combined effect of lack of moisture and substrate type S3,  $I_{CH}$  is an indicator variable equal to 1 when the moisture treatment is present, but zero otherwise, and  $I_{S1}$  is an indicator variable equal to 1 when substrate 1 (S1) treatment is present, but zero otherwise. Further  $I_{S2}$  is an indicator variable equal to 1 when substrate 2 (S2) treatment is present, but zero otherwise, and  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the parameters to be estimated.

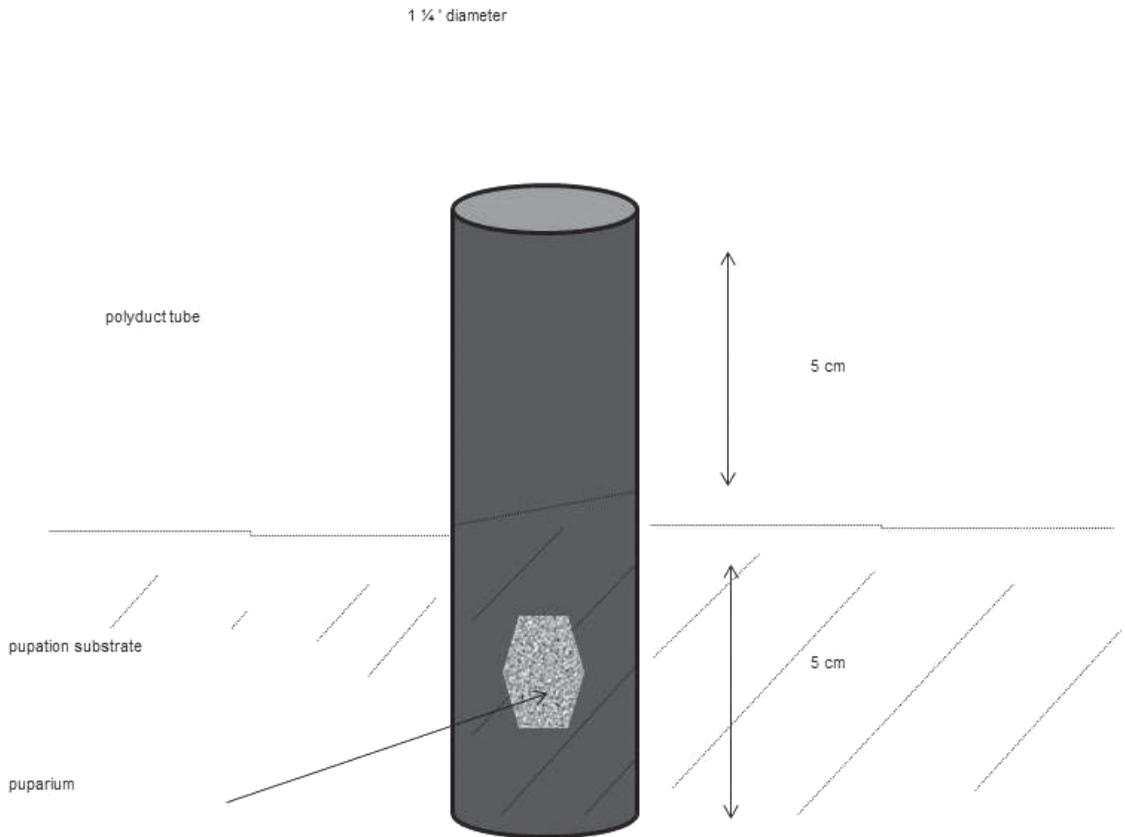


Fig. 1. Characteristics of *Comadia redtenbacheri* pupation chambers allowing for control of individual pupae. These chambers had a layer of soil at the bottom and were supported in plastic trays.

## 2. Effect of Larval Manipulation and Moisture on Adult Emergence

Six trays were set up each with 50 “commercial” larvae placed individually in the polyduct tubes. Half of the trays received water sprayed in the same way as in the above experiment. This protocol was repeated with “no manipulation” larvae. The pupation substrate was soil from the areas of agave that hosted the redworm. In this experiment also the numbers of emerged adults were recorded and used as the dependent variable analyzed with logistic regression (PROC GENMOD) to determine the odd-ratio of adult emergence vs. total larvae by the effect of the presence of moisture and manipulation.

The basic structure of the linear model use in the logistic regression structure was:

$$\mu = \beta_0 + \beta_1 I_{CM} + \beta_2 I_{CH}$$

where  $\mu$ , is the linear model,  $\beta_0$  is the intercept representing the combined effect of lack of moisture and manipulation,  $I_{CM}$  is an indicator vari-

able equal to 1 when manipulation factor is present, but zero otherwise,  $I_{CH}$  is an indicator variable equal to 1 when moisture is present, but zero otherwise, and  $\beta_1$  and  $\beta_2$  are the parameters to be estimated.

## 3. Pupation, Adult Emergence, and Proportion of Sexes by Larval Weight Class

The experiment was set up by placing fifth instar larvae weighing 0.30 to 0.39 g on the same tray, and those of sixth and seventh instars weighing 0.40 g or more on another tray. Each tray with 50 larvae was an experimental unit and was replicated 4 times. All trays were irrigated. New soil was used as substrate in half of the trays, and reused soil in the other half of the trays to determine whether reused soil affects pupal development. The results for adult emergence by larval weight and soil condition were analyzed by logistic regression (PROC GENMOD). The factors analyzed related to the odd-ratio of total adults emerged vs. total larvae taking as factors the larval weights from 0.30 a 0.39 g and 0.40 g

or more. The data on proportion of sexes with respect to weight class were examined with the chi squared test ( $\chi^2$ ).

The basic structure of the linear model used in the logistic regression structure was:

$$\mu = \beta_0 + \beta_1 I_{p_{0.30 \text{ a } 0.39 g}} + \beta_2 I_{SN}$$

where  $\mu$ , is the linear model,  $\beta_0$  is the intercept representing the combined effect of weight > 0.40 g and reused soil,  $I_{p_{0.30 \text{ a } 0.39 g}}$  is an indicator variable equal to 1 when the larval weight was 0.30 a 0.39 g, but zero otherwise,  $I_{SN}$  is an indicator variable equal to 1 when the new soil is present, but zero otherwise and  $\beta_1$  and  $\beta_2$  are the parameters to be estimated.

Through destructive sampling we observed that the pupal period lasts approximately 5 months, and in the first month the insects remain as larvae within cocoons (Llander-Cázares et al. 2007). The larvae initiated pupation between 25 Sep and 2 Oct 2010. In total, 4 samplings were done: #1 (14 Dec) at 2.5 months after placing the larvae under conditions of pupation; #2 and #3, after 3 and 4.5 months (14 Jan and 14 Feb 2011, respectively), and #4 at 5 months (3 Mar 2011). Each sampling consisted in selecting at random from different trays belonging to the 3 previous experiments, one tube with a cocoon from each tray, i.e., a total of 41 cocoons. In undeveloped individuals, the stage (larval or pupal) at which growth had ceased was observed and the possible cause (fungi, bacteria or parasites) was determined.

4. Effect of Larval Manipulation, Type of Substrate and Moisture on Adult Emergence

We used 16 trays, half with the “commercial” larvae and the other half with the “no manipulation” larvae. Within each test 4 trays had substrate type S1 (soil from agave areas where the larvae were collected) and the other 4 had substrate type S4 (50:50 mixture of S1 and vermiculite). Moisture was provided to 2 trays with S1 and to 2 trays with S4. Finally, the number of

emerged adults per treatment was recorded and the results were analyzed with logistic regression (PROC GENMOD).

The basic structure of the linear model used in the logistic regression structure was:

$$\mu = \beta_0 + \beta_1 I_{CM} + \beta_2 \beta_{CH} + \beta_3 I_{S4}$$

where  $\mu$ , is the linear model,  $\beta_0$  is the intercept representing the combined effect of lack moisture and manipulation and Soil type S1,  $I_{CM}$  is an indicator variable equal to 1 when the moisture treatment is present, but zero otherwise,  $I_{CH}$  is an indicator variable equal to 1 when moisture is present, but zero otherwise,  $I_{S4}$  is an indicator variable equal to 1 when Soil 2 (S2) treatment is present, but zero otherwise, and  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the parameters to be estimated.

The formulation used in all the experiments allowed us to evaluate the effect of the treatments based on the significance of the additive parameters in relation to the base parameter ( $\beta_0$ ). When the other additive parameters become statistically significant ( $\alpha \leq 0.05$ ), it was possible to conclude that the odds-ratio comparison among treatments is statistically different.

The  $\chi^2$  test is used in observation studies, in which it is common to obtain data collected simultaneously for 2 variables classified by their provenance in order to test the hypothesis that the frequency in the categories of one variable is independent of the frequency in the second variable (Herrera & García 2010). Analysis of these tests was done by statistical software SAS Institute (2008). Using these models, it was possible to identify in the 3 experiments, which conditions were better for the development of the insect.

RESULTS AND DISCUSSION

1. Evaluation of Substrates and Moisture on Pupation and Adult Emergence

Table 1 shows the results on pupae confined in chambers with different types of substrates.

TABLE 1. EFFECT OF VARIOUS SUBSTRATES AND SUPPLEMENTAL MOISTURE ON ADULT EMERGENCE OF *COMADIA REDTENBACHERI*.

Parameter	Independent Variable	DF	Estimated value	Parameter error	95% CI	P-value	Odds ratio
Intercept	$\beta$ (No moisture + S3 <sup>3</sup> )	1	-1.6926	0.2296	-2.1611 -1.2578	<0.0001	
Moisture	$I^0$ (With moisture)	1	-0.3624	0.2271	-0.8061 0.0863	0.1166	0.69
Substrate	$I_{S1}^{CH}$	1	0.0342	-0.1983	-0.1983 0.8936	0.2171	1.41
Substrate	$I_{S2}^{S1}$	1	0.0769	-0.4921	-0.3963 0.726	0.5695	1.17

<sup>1</sup>Soil from areas of agave where larvae were collected  
<sup>2</sup>S2: 75:25 mixture soil and vermiculite  
<sup>3</sup>S3: peat moss. Linear model =  $\mu = \beta_0 + \beta_1 I_{CH} + \beta_2 I_{S1} + \beta_3 I_{S2}$

There were no significant differences in numbers of emerged adults among substrates, nor did moisture have a significant effect (an 18% average odds-ratio of emerged vs. total). It can thus be deduced that the agave redworm has a high capacity to pupate in soil with different texture and structure, even in the absence of supplemental moisture. This should greatly facilitate management of the insect under artificial conditions, although it might be helpful to continue experimentation with other types of substrates. According to Madrigal (2001), moisture should be kept at 75%, since low moisture contents produce desiccation, while high moisture contents favor fungal and bacterial growth. Soto-Manitiu et al. (1997) postulated that for *Anastrepha obliqua* (Diptera: Tephritidae) soil moisture affected the duration of its pupal period. Their study found, however, that it is atmospheric relative humidity that has a strong influence on adult emergence.

2. Effect of Larval Manipulation and Moisture on Adult Emergence

According to the results shown in Table 2, the probability of adult emergence is significantly higher from “no manipulation” larvae + no moisture, while emergence from “commercial” larvae drops from 27% to 18%. The odds ratio of commercial larvae to no manipulation larvae suggests that the probability of adult emergence is 40% lower from the commercial larvae. In insect rearing, it has been determined that direct handling

of the larvae must be minimal during their entire development, since handling causes stress (Madrigal 2001). Of the “no manipulation” larval samples, 15.3% were damaged, diseased or parasitized, while in the “commercial” sample the percentage was 13.2%; this could indicate that the gatherers tended to differentially select healthy undamaged larvae, although imperceptible damage and mistreatment during distribution and sale were not considered.

In Table 2, it can be seen that moisture content was somewhat significant (0.0662) in increasing adult emergence from 24% and 35% in “commercial” and “no manipulation” larvae, respectively; a result that differs from the previous experiment in which moisture was not significant and had negative values. In contrast, Véjar (2004) reported this factor as one of the most important in rearing insects. This would point to the need for additional investigations on the effect of watering the pupation trays.

3. Pupation, Adult Emergence and Proportion of Sexes by Larval Weight

Larvae obtained from agave rhizomes are of diverse sizes, and it is important to know the minimum weight at which they can still transform into pupae. In this study, it was determined that larvae weighing as little as 0.30 g can successfully metamorphose into pupae and adults. Indeed these small larvae were not significantly different from heavier larvae in their capacity

TABLE 2. EFFECT OF ROUGHNESS OF HANDLING LARVAE AND SUBSTRATE MOISTURE ON ADULT EMERGENCE OF *COMADIA REDTENBACHERI*.

Parameter	Independent Variable	DF	Estimated Parameter			P-value	Odds ratio
			value	error	95% CI		
Intercept	$\beta_0$ (no commercial manipulation + no moisture)	1	-0.9609	0.1665	-1.4045 -0.7603	<0.0001	
Manipulation	$I_{CM}$ (commercial manipulation)	1	-0.5082	0.1958	-0.875 -0.1175	0.0094	0.60
Moisture	$I_{CH}$ (With moisture)	1	0.3583	0.1951	-0.0276 0.7267	0.0662	1.43

The pupation substrate was soil from the areas of agave that hosted the redworm Linear model =  $\mu = \beta_0 + \beta_1 I_{CM} + \beta_2 I_{CH}$ .  
 Note: Commercial operations to extract larvae from underground rhizomes of agave plants are performed rapidly, and such rough handling may be stressful to the larvae. In contrast laboratory personnel exercised great care not to stress larvae being extracted from rhizomes.

TABLE 3. *COMADIA REDTENBACHERI* PUPATION CAPACITY AND ADULT EMERGENCE RELATIVE TO LARVAL WEIGHTS OF 0.3-0.39 G AND > 0.4 G, AS WELL AS NEW VS. RE-USED SUBSTRATE.

Parameter	Independent variable	DF	Estimated Parameter			P-value	Odds ratio
			value	error	95% CI		
Intercept	$\beta$ (> 0.4 g+ Re-used substrate)	1	-2.0661	0.1780	-1.4045 -0.7603	< 0.0001	
Weight	$I^0$ (0.3-0.39 g)	1	-0.2899	0.2613	-0.875 -0.1175	0.2672	0.74
Substrate	$I^{SN}$ (New substrate <sup>1</sup> )	1	0.4276	0.2627	-0.0276 0.7267	0.1036	1.53

<sup>1</sup>Soil used for the first time as a substrate for pupation. Linear model =  $\mu = \beta_0 + \beta_1 I_P + \beta_2 I_{SN}$ .

to pupate, as observed in the logistic regression analysis (Table 3). It should be mentioned that the weight range is small, and this could be the reason that there are no significant differences. It would be necessary to perform tests with smaller larvae to determine the minimum weight at which they can still continue to pupate. Nolasco et al. (2002) considered that the larvae extracted from the plant by collectors could be too immature to pupate. Camacho et al. (2003, 2005) induced pupation of larvae, which—based on the size of the cephalic capsule—were considered to be in the last 2 instars; these workers assumed that these larvae would be the only ones capable of completing the pupation process. However, the earlier instars that they used remained several weeks in boxes with soil, but the authors did not indicate whether they pupated.

The statistical analysis showed no significant variability in adult emergence by effect of a recently prepared pupation substrate or a reused substrate (Table 3). Re-use of substrate would be an economic advantage in rearing this insect. Madrigal (2001) reported that for some species, re-use of the pupation substrate requires it to be sterilized, as in the case of the parasitoid *Teleonomus alsophilae* Vierek (Hymenoptera: Scelionidae).

By means of a contingency table (Table 4), we found that lower weight (0.30-0.39 g) fifth instar larvae are as capable of pupating as the heavier weight (> 0.40 g) sixth and seventh instars. Also

we determined that there is a certain relationship between weight and sex of emerged adult (Table 4), as occurs in many Lepidoptera species (Allen et al. 2011). The smaller-sized larvae tend to develop into male adults (41.5%), while the larger larvae tend to develop into female adults (29.1%), although some males, as larvae, also weighed > 0.40 g (Table 4). Statistical significance for the  $\chi^2$  test was < 0.0001. Thus, if a sample that includes larvae of both weight ranges is taken, the probability that adults of both sexes will emerge increases.

Destructive Sampling and Progression of Metamorphosis

When larvae were placed in the pupation chamber, it was observed that most dug into the soil, and that most of those that remained on the surface died. There were also cases in which larvae having dug into the substrate later returned to the surface and most of these did not survive.

With the first destructive sampling (Fig. 2), we determined that the larvae inside their cocoons remained in the larval stage for 2.5 months. At the second sampling date, prepupae were found and 7.3% of the larvae were inactive. The percentage of inactive larvae increased to 17% in Feb. On 3 Mar 2012, five months after pupation induction, 12% had become pupae and 14.6% were prepupae (less mobile, lighter color and shorter body). Once we had found pupae, we waited almost a month

TABLE 4. *COMADIA REDTENBACHERI* ADULT EMERGENCE AND PROPORTION OF FEMALES AND MALES RELATIVE TO LARVAL WEIGHT.

Number / Percentage	Weight (g)	Female	Male	Total
	0.30- 0.39	6	37	43
		6.74	41.57	48.31
	> 0.40	26	20	46
		29.21	22.47	51.69
	Total	32	57	89
		35.96	64.04	100

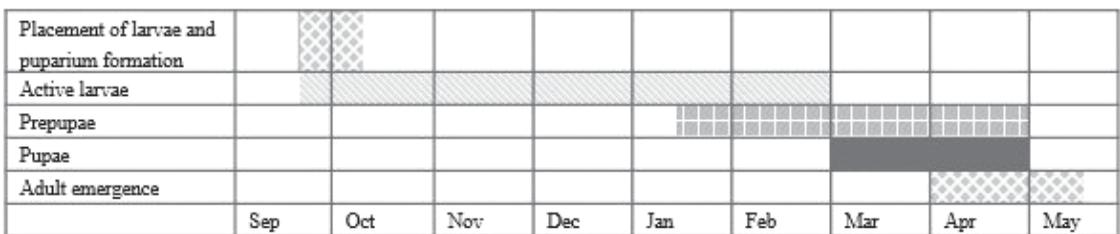


Fig. 2. Degree of progress, in months, toward transformation of *Comadia redtenbacheri* larvae weighing 0.30 g or more into adults.

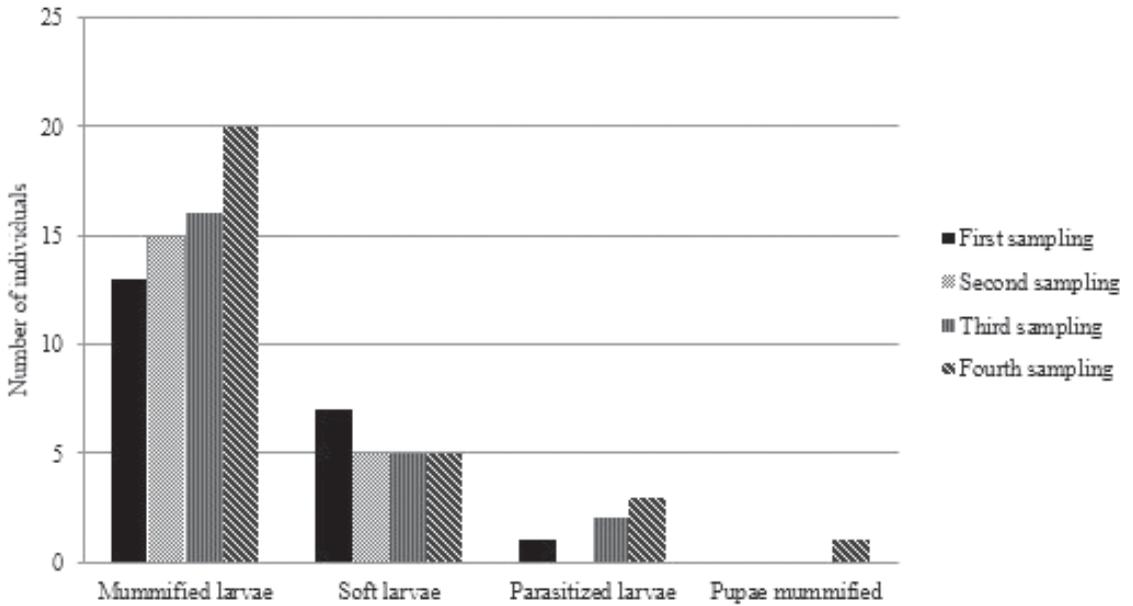


Fig. 3. Number of *Comadia redtenbacheri* individuals that did not develop to adults because of possible desiccation, disease or parasitism.  $N = 164$  cocoons taken at random in the 4 destructive samplings during the period of pupation.

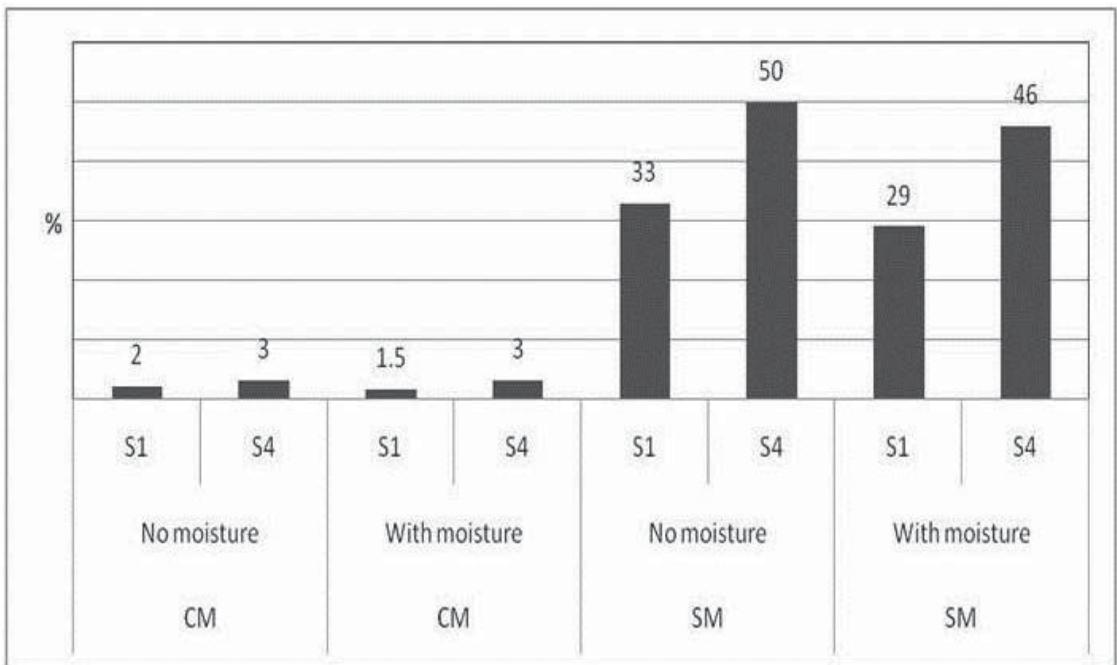


Fig. 4. Histogram of *Comadia redtenbacheri* adult emergence percentages by treatment. CM: commercial manipulation, SM: No commercial manipulation, S1: Soil from areas where larvae were collected, S4: 50:50 mixture soil:vermiculite.

TABLE 5. EFFECT OF SUBSTRATES, MANIPULATION AND MOISTURE ON *COMADIA REDTENBACHERI* ADULT EMERGENCE.

Parameter	Independent Variable	DF	Estimated Parameter		95% CI	P-value	Odds ratio
			value	error			
Intercept	$\beta$ (SM <sup>1</sup> +no moisture+S1 <sup>2</sup> )	1	-0.7068	0.1772	-1.0599 -0.3639	<0.0001	
Manipulation	I <sup>0</sup> (Commercial manipulation)	1	-3.3031	0.3382	-4.0354 -2.6962	<0.0001	0.03
Moisture	I <sup>CM</sup> (With moisture)	1	-0.1559	0.1975	-0.5441 0.2310	0.4301	0.85
Substrate	I <sup>CH</sup> <sub>S4</sub>	1	0.7304	0.1990	0.3430 1.1240	0.0002	2.07

<sup>1</sup>SM: no commercial manipulation.

<sup>2</sup>S1: Soil from areas of agave where larvae were collected.

<sup>3</sup>S4: 50:50 mixture soil and vermiculite. Linear model=  $\mu = \beta_0 + \beta_1 I_{CM} + \beta_2 I_{CH} + \beta_3 I_{S4}$ .

for the first adults to emerge. The period of adult emergence extended to 24 May of the same year (Fig. 2).

Once the cocoon was formed, larvae required approximately 5 months to become adults. The dead *C. redtenbacheri* found during destructive sampling were classified and both the development stage at which the transformation process ceased and the possible cause of death were recorded (Fig. 3). We found that 39.6% of the individuals died in the larval stage because of the entomopathogenic fungi, *Beauveria* spp., which causes hardening and mummification. *Beauveria* has been reported as the cause of death in several insect species (Castillo et al. 2009). For *Anastrepha obliqua* (Macquart) (Tephritidae), Chaverri et al. (1999) determined that a percentage of pupae do not reach emergence because of damage by fungi and parasitoids. We found bacterial infection to be the second most prevalent cause of death. This was manifested in the soft consistency and characteristic odor of infected larvae. The third cause of mortality was parasitoids. Also, one mummified pupa was found. Approximately 1 month before and during adult emergence, the parasitoids of *C. redtenbacheri* reported by Zetina et al. (2009, 2011, 2012), emerged out of the cocoons. First came the tachinid *A. texana* and, at the end of the period, the ichneumonid, *L. fascipennis*.

#### 4. Effect of Larval Manipulation, Substrate Type and Moisture on Adult Emergence

The data shown in Table 5 corroborate that manipulated larvae had less vigor to complete the life cycle than the non manipulated larvae. The 50:50 mixture of soil and vermiculite (S4) was more easily managed and cheaper than S2 substrate used in 2010, which had 25% less vermiculite. S4 was significantly different from the collection site soil (S1), and it increased pupation by a 2.07 odds-ratio. It was also shown that the pupation process can occur without supplemental moisture. Based on these results, the use of larvae for breeding stock that have not been handled much and a 50:50 mixture of soil with vermiculite for pupation substrate are recommended.

Figure 4 shows the likelihoods of adult emergence in percentages, according to the relationships among the treatment variables. The highest percentages are found in the treatments comprising non-manipulated larvae. The effect of the substrate (S4) raises the likelihood of adult emergence to 50%, if moisture is not present. In this experiment the effect of manipulation was very important since the reference case, which is the intercept, indicates 33% adult emergence for non-manipulated larvae, while that of manipulated larvae is only 1.5%.

Because *C. redtenbacheri* is a borer, the study of its biology and behavior is complicated and its rearing in the laboratory is difficult. Forchler & Nordin (1989) state that the study of wood borers is often limited because methods for rearing a suitable number of insects are lacking. In the case of the maguey redworm, until now the only way to have sufficient larvae for study was to acquire wild populations. Collection of these populations is difficult because the host agave rhizome must be destroyed to extract the larvae. Once collected, the larvae are kept in overcrowded receptacles until they are used, causing contamination and deterioration. Consequently, the number of healthy individuals that could serve as breeding stock is reduced. Zetina et al. (2011) reported a model for predicting parasitism in *C. redtenbacheri* larvae and they mention the need to find procedures to avoid losses of material, costs and time during acquisition of breeding stock. They comment that a redworm larva in good condition will be more likely to reach adulthood and reproduce. The results obtained in this study can improve methods for obtaining adults through selection of the larvae by provenance, health and size, as well as for conservation of the pupae.

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