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A new substitute host and its effects on some biological properties of *Ooencyrtus kuvanae*

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Abstract

Lymantia dispar (L.) (Lepidoptera: Lymantriidae), commonly known as the gypsy moth, is a serious forest pest, and beneficial insects are particularly important for reducing its population numbers. *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) is an arrhenotokous, solitary egg parasitoid of *L. dispar*. In this study, we evaluated a new substitute host, *Philosamia ricini* (Danovan) (Lepidoptera: Saturniidae) for *O. kuvanae*. We investigated some of the biological effects of *O. kuvanae* on *P. ricini* eggs. In this context, the importance of the age of the female parasitoid (1, 3 or 5 days old), host age (1–2 and 3–4 days old) and host number (40, 60 and 80 host eggs) were examined under laboratory conditions (25 ± 1 °C, 65 ± 5% relative humidity and a 16 : 8 h photoperiod [light : dark]). The highest rate of offspring production (89.90%) occurred with 40 (1–2-day-old) host eggs and 5-day-old females. The mean developmental period ranged from 16.5 ± 0.08 days to 18.7 ± 0.08 days. The mean lifespan of the parasitoid was 51.10 ± 1.1 ($n = 60$) days with bio-honey and 3.92 ± 0.14 ($n = 60$) days without food. The mean fecundity was 68.88 ± 3.22 offspring/female. Peak adult emergence occurred between 2 and 9 days. The mean oviposition and mean post-oviposition periods of the female parasitoid were 22.76 ± 1.37 days and 13.64 ± 1.40 days, respectively. *O. kuvanae* was reared for more than ten generations on the eggs of *P. ricini*. Based on our findings, *P. ricini* can be used to rear *O. kuvanae* for the biological control of *L. dispar*.

Keywords: *Lymantia dispar*, *Philosamia ricini*, *Ooencyrtus kuvanae*, biology

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Introduction

A forest ecosystem is a complex unit of biodiversity, and its components include plants, animals, insects, microorganisms and their interactive relationships (Hunter, 1999). Due to their toxic effects on many beneficial organisms, chemical pesticides should not be used for insect pest control in sustainable forest ecosystems. Furthermore, the overuse of pesticides for pest control may result in the development of potential resistance to insecticides by the pest insects being targeted (Sánchez-Bayo *et al.*, 2011). Thus, green pest-control methods such as microbial control and traps and beneficial insects should be used to replace pesticides.

Lymantria dispar (L.) (Lepidoptera: Lymantriidae), the gypsy moth, is a defoliator of mainly forest trees (Gould *et al.*, 1990). It is of Eurasian origin and has a range that covers Europe, Africa, and North America (Keena *et al.*, 2008). Gypsy moth larvae are known to feed on over 500 plant species within 73 families (Lance, 1983; Liebhold *et al.*, 1995; Mrdaković *et al.*, 2013). The larvae can cause economic damage and reduce forage production. The greatest impact of gypsy moths is the physiological stress in trees caused by defoliation (Humble & Stewart, 1994; Papadopoulou *et al.*, 2009).

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Biological control is an alternative approach to reducing populations of *L. dispar*, using natural enemies. *Bacillus thuringiensis*-based insecticides are in widespread use because of their specific toxicity against certain pests in the larval stage (Höfte & Whiteley, 1989). The microbial insecticide *Bacillus thuringiensis var. kurstaki* is often used to manage *L. dispar* (McCullough *et al.*, 1999; Fabel, 2000). However, parasitoids play an important role in the biological control of *L. dispar*, and several European hymenopteran parasitoids of the gypsy moth have been established (e.g., *Ooencyrtus kuvanae* [Howard] [Encyrtidae] *Anastatus japonicus* Ashmead [=disparis Rushka] [Eupelmidae], *Cotesia melanoscelus* Ratzeburg [Braconidae], *Phobocampe disparis* [Viereck] [Ichneumonidae], *Monodontomerus aereus* Walker [Torymidae], *Brachymeria intermedia* [Nees]).

O. kuvanae (Howard) is a small encyrtid egg parasitoid that serves as a potential biological control agent of *L. dispar*. *O. kuvanae* was originally known to exist only in Japan, but now it is found to have nearly a Holarctic distribution. This parasitoid is an arrhenotokous and multivoltine species (Tadic & Bincev, 1959; Brown, 1984). The egg stage of *L. dispar* has a very long period, which can be used by *O. kuvanae* to go through several generations, each contributing to augmented parasitism rates in the field (Hofstetter & Raffa, 1998; Wang *et al.*, 2013). In addition, *O. kuvanae* can adapt to several different environmental conditions and is an abundant species (Brown, 1984). However, it is not possible to rear this parasitoid on its natural host under laboratory conditions, because its natural host, *L. dispar*, is an univoltine species; additionally, the egg masses and urticacious hairs on the larvae of this host may cause allergic reactions in humans (Fabel, 2000; McCullough & Bauer 2000; Tong *et al.*, 2000). There are three main methods of rearing parasitoids, namely, on a natural host, on a substitute host and on an artificial diet (Consoli *et al.*, 2000). Natural enemy rearing on substitute hosts is a determining factor for the success of many biocontrol programs, because this rearing option reduces production costs and increases the viability of large-scale use of the beneficial insect (Parra, 1997).

In this study, *Phylosamia ricini* Donovan (Lepidoptera: Saturniidae) was selected as a new substitute host. *P. ricini* eggs have been used for the laboratory rearing of a number of parasitoids such as *Trichogramma chilonis* Ishii, *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) and *Anastatus japonicus* (Hymenoptera: Eupelmidae) (Pu *et al.*, 1988; Liu *et al.*, 1998). The host plants of *P. ricini*, *Ligustrum vulgare* (Lamiales: Oleaceae) and *Ailanthus* spp., facilitate the rearing of this lepidopterous species in the laboratory (Saito, 1998; Osanai *et al.*, 2000; Tiradon *et al.*, 2013). Females lay many eggs during their short lifespan (approximately 250 eggs per female); they are not subject to diapause and their eggs are large (1656.5 × 1143 μm^2) (Tunca *et al.*, 2015).

Host quality is a significant cause for the success of parasitism by parasitoid biocontrol agents. Host size (particularly for solitary parasitoids), host plant, host species and host age can affect host quality (Vinson & Iwantsch, 1980; King, 1987; Godfray, 1994; Campan & Benrey, 2004; Shuker & West, 2004; Ueno, 2005). Host age is an important factor influencing host acceptance and host suitability for the parasitoid egg (Vinson & Iwantsch, 1980; Zhou *et al.*, 2014). Furthermore, the age of the female parasitoid is a determinant of reproductive rate and can affect parasitism (Amalin *et al.*, 2005; Aung *et al.*, 2010; Pizzol *et al.*, 2012). In this study, the age of the host and the age of the female parasitoid were investigated, with the aim of evaluating the new substitute host, *P. ricini*. We investigated the biological characteristics (offspring production ratio, development time, longevity, and fecundity) of *O. kuvanae* reared on eggs of this host. The establishment of new laboratory rearing methods of *O. kuvanae* on *P. ricini* will contribute to the laboratory rearing of this parasitoid.

Materials and methods

This study was performed at the INRA-PACA Mediterranean Forest and Entomology Unit, Laboratory of Biological Control, Antibes, France.

Rearing the host P. ricini

P. ricini eggs were collected daily in a Petri dish (5 cm) and kept inside an incubator at 25 ± 1 °C, 65 ± 5% relative humidity (RH), and a photoperiod of 16 : 8 h (light : dark [L : D]). Newly hatched larvae were transferred to plastic boxes (26 × 12 × 7 cm^3) and were fed every day with fresh privet leaves, *L. vulgare* (Lamiales: Oleaceae). Different larval stages were reared in separate boxes and observed until pupation. Just after the pupal stage, individual pupae were shifted to adult rearing cages (30 × 39 × 30 cm^3). This process was repeated on a daily basis.

Rearing the parasitoid O. kuvanae

The *O. kuvanae* colony was obtained from the parasitized eggs of *L. dispar* collected from the fields in Arbois-Avignon. Adult parasitoids were reared in glass tubes (1 × 7 cm^2) and maintained in an incubator at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16 : 8 h (L : D). A drop of bio-honey was offered at 2-day intervals as a food source for adult parasitoids. The egg masses of *P. ricini* were collected and exposed daily to the mated female parasitoid. Then the offspring were allowed to emerge. Female and male parasitoids were collected at emergence. After mating, 90 females were kept to reach the different ages needed for the experiments. The remaining adult parasitoids were used to establish parasitoid cultures under laboratory conditions.

Experimental techniques

We tested the effects of age and number of *P. ricini* eggs and age of *O. kuvanae* females on offspring production ratio and development time. For this purpose, we used a 2 × 3 × 3 factorial design. There were two levels of treatment according to the age of *P. ricini* (1–2 days old and 3–4 days old), three levels of treatment for the number of *P. ricini* (40, 60, and 80 eggs), and three levels of treatment for the age of *O. kuvanae* (1, 3 and 5 days old). Furthermore, 40, 60 or 80 unparasitized eggs of *P. ricini* (either 1–2 or 3–4 days old) were exposed in a test tube (1 × 7 cm^2) to single mated *O. kuvanae* females (1, 3 or 5 days old), with a drop of honey, for parasitism. The bottoms of each test tube were covered with cotton. A total of 180 host eggs per treatment and a total of 1080 host eggs per replication were used. The experiments were performed in five replicates. The emergence of adult parasitoids (at regular intervals of 24 h) and the dates of emergence of *O. kuvanae* adults were recorded to determine the offspring production ratio and development time. In addition, the longevity of the adult parasitoids was investigated under dietary (bio-honey)

Table 1. Results of a GLM analysis of the offspring production ratio of *Ooencyrtus kuvanae*.

Source of variation	Degrees of freedom	Sum of square	F value	P value
Parasitoid age	2	13631.0	297.41	$P < 0.001$
Host number	2	6195.9	135.19	$P < 0.001$
Host age	1	72.6	3.17	0.079
Parasitoid age × host number	4	647.4	7.06	$P < 0.001$
Parasitoid age × host age	2	37.4	0.82	0.446
Host number × host age	2	8.8	0.19	0.826
Parasitoid age × host number × host age	4	26.0	0.28	0.888
Error	72	1649.9		

Table 2. Effects of parasitoid age and host number on the offspring production ratio of *Ooencyrtus kuvanae* (mean ± standard error).

Parasitoid	Host number		
age (days)	40	60	80
1	47.20 ± 0.018 A[1] b[2] $n = 10$	36.50 ± 0.015 A c $n = 10$	27.60 ± 0.019 B c $n = 10$
3	76.90 ± 0.023 A a $n = 10$	56.70 ± 0.017 B b $n = 10$	47.10 ± 0.014 B b $n = 10$
5	89.90 ± 0.013 A a $n = 10$	79.30 ± 0.020 A a $n = 10$	62.30 ± 0.015 B a $n = 10$

[1] Means in each row followed by the same capital letter do not differ statistically.

[2] Means in each column followed by the same lowercase letter do not differ statistically.

and non-dietary conditions. Longevity was measured from adult emergence until death. The experiment was performed in triplicate. A single mated *O. kuvanae* female (1-day old) reared from the eggs of *P. ricini* was introduced into a test tube (1×7 cm^2) with 15 *P. ricini* eggs. Bio-honey was supplied as a nutrient for the female parasitoid. The *P. ricini* eggs were replaced daily with new batches of 15 eggs until the female died. The previous eggs were transferred to an incubator (25 ± 1 °C, 65 ± 5% RH and a photoperiod of 16 : 8 h [L : D]). Seventeen female parasitoids were tested, and we measured the realized fecundity and the means of the oviposition period and the post-oviposition period.

Data analysis

The offspring production ratio and development time were analyzed using a general linear model with the age of the hosts, number of hosts and the age of the female parasitoid as factors. Percentage data were normalized using an arcsine transformation ($p' = \text{arcsine}\ \sqrt{p}$; Zar, 1999). Longevity was analyzed using a two-sample t test (Minitab Release 14; SAS Institute, 2003; McKenzie & Goldman, 2005). The corresponding means (±standard error) for the oviposition and post-oviposition periods were calculated for the realized fecundity.

Results

There was a significant impact ($P < 0.001$) of both parasitoid age and host number on the offspring production ratio (table 1). The highest and the lowest offspring production percentages were obtained from a 5-day-old female with 40 host eggs (89.90%) and a 1-day-old female with 80 host eggs (27.60%) (table 2). Three factors (parasitoid age × host number × host age) showed a significant interaction ($P < 0.001$) on the development time of *O. kuvanae* (table 3). The minimum and maximum mean development times were 16.5 ± 0.08 days and 18.7 ± 0.08 days, respectively (table 4). Although these development times do not seem to differ much, a significantly shorter development time was observed in young (1–2-day-old) host eggs. The mean lifespan of adults was 51.10 ± 1.1 days with honey and 3.92 ± 0.14 days without honey (t60 = −42.05, $P < 0.000$). The mean number of offspring developed from the eggs laid by a single female reared on *P. ricini* (realized fecundity of female *O. kuvanae*) was 68.88 ± 3.22. Furthermore, the means of the oviposition and post-oviposition periods were 22.76 ± 1.37 days and 13.64 ± 1.40 days, respectively. Peak adult emergence occurred between 2 and 9 days. The emergence of the adults continued for about 3 weeks (fig. 1).

Discussion

We first determined some of the biological effects of the egg parasitoid *O. kuvanae* on a new substitute host, *P. ricini*. Substitute hosts are used to either decrease rearing costs or increase performance in rearing of parasitoids, and a careful selection of substitute hosts is important in biological control programs (Fedde *et al.*, 1982); nevertheless, the suitability of these alternative hosts can vary greatly for a polyphagous parasitoid (Hoffmann *et al.*, 2001). Initially, basic biological studies should be performed taking into account various factors (e.g., host age, host number and parasitoid age).

The percentage of offspring that emerged refers to the suitability of the host for insect parasitoid development (El Sharkawy, 2011). In our study, the optimal offspring production rate was obtained from 40 host eggs and 5-day-old females. Our study demonstrates that the age of the host egg has no effect on the parasitoid's offspring production rate. *O. kuvanae* females were able to parasitize 1–2-day-old and 3–4-day-old eggs of *P. ricini*. The age of the host is one of the most important factors determining host acceptance by parasitoids (Vinson, 1985). According to Strand (1986) and Vinson (1998), hymenopter parasitoids can parasitize and develop on all host developmental stages, as they are able to adapt to a range of host conditions. However, the preference for young host eggs is important because the development of parasitoid offspring is influenced by the nutritional quality of the host eggs. Egg parasitoids often choose young or intermediately aged host eggs for parasitism (Reznik & Umarova, 1990; Monje *et al.*, 1999).

Researchers have also found that old host eggs (≥4 days) are less suitable for some *Ooencyrtus* species, although

Table 3. Results of a GLM analysis of the development time of *Ooencyrus kuvanae*.

Source of variation	Degrees of freedom	Sum of square	*F* value	*P* value
Parasitoid age	2	27.485	14.11	$P < 0.001$
Host number	2	20.052	10.29	$P < 0.001$
Host age	1	992.218	1018.48	$P < 0.001$
Parasitoid age × host number	4	86.649	22.24	$P < 0.001$
Parasitoid age × host age	2	21.104	10.83	$P < 0.001$
Host number × host age	2	12.242	6.28	0.002
Parasitoid age × host number × host age	4	27.103	6.96	$P < 0.001$
Error	72	2885.612		

Table 4. Variation in development time (mean ± standard error) of *Ooencyrtus kuvanae* with parasitoid age, host number, and host age.

	Host number					
Parasitoid age (days)	40 (1–2 days)	60 (1–2 days)	80 (1–2 days)	40 (3–4 days)	60 (3–4 days)	80 (3–4 days)
1	16.7 ± 0.08 CB[1] a[2] $n = 95$	16.5 ± 0.08 CB a $n = 106$	17.2 ± 0.09 AB a $n = 110$	17.8 ± 0.07 AB a $n = 93$	18.3 ± 0.1 A a $n = 113$	18.7 ± 0.08 A a $n = 110$
3	17.5 ± 0.06 AB b $n = 147$	17.2 ± 0.07 AB a $n = 164$	16.7 ± 0.06 B a $n = 183$	18.3 ± 0.09 A a $n = 160$	17.9 ± 0.06 A a $n = 176$	18.1 ± 0.06 A b $n = 192$
5	16.8 ± 0.05 B a $n = 178$	16.7 ± 0.06 B a $n = 234$	16.9 ± 0.07 B a $n = 245$	17.9 ± 0.06 AB a $n = 181$	17.9 ± 0.07 AB a $n = 241$	18.0 ± 0.07 A b $n = 252$

[1] Means in each row followed by the same capital letter do not differ statistically.
[2] Means in each column followed by the same lowercase letter do not differ statistically.

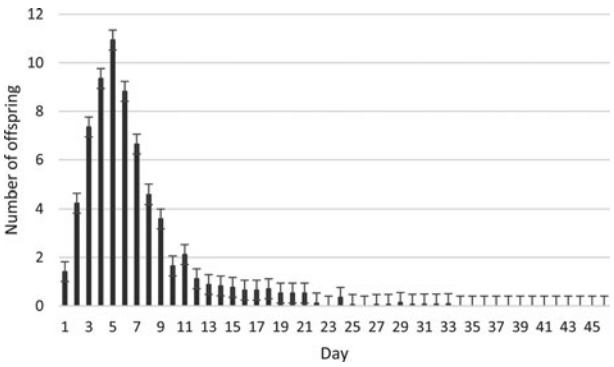

Fig. 1. Daily offspring number (mean ± SE) of *Ooencyrthus kuvanae* over the lifetime.

Ooencyrtus females are able to adapt to any host egg (Nechols *et al.*, 1989; Takasu & Hirose 1993; Hofstetter & Raffa 1998). Our results are in agreement with those of Zhao *et al.* (2013), who found that all ages (2, 3, 4, 5, 6 and 7 days old) of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) pupae can be successfully parasitized by the parasitoid *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae). Zhou *et al.* (2014) reported that *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) can successfully develop in all ages of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) eggs. Pak *et al.* (1986) and Jacob *et al.* (2006) showed that old host eggs do not have any negative effect on parasitoid preference or offspring fitness. On the other hand, King (1998) demonstrated that the number of offspring decreases with increasing host age (1-, 2-, 3-, 4- and 5-day-old housefly pupae) for the pupal parasitoid *Spalangia cameroni* (Wiki) (Hymenoptera: Pteromalidae). Da Rocha *et al.* (2006) observed that offspring emergence of the egg parasitoid *Gryon gallardoi* (Hymenoptera: Scelionidae) decreases with increasing host age. Furthermore, different ages (1, 2 or 3 days old) of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) eggs affect the percentage emergence of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) (Peñaflor *et al.*, 2012). We conclude that the effects of host age vary according to parasitoid species.

In our study, we observed minimal decreases in development time with increases in the age of *P. ricini* eggs. Several studies have found that development time varies with host age in many parasitoids, such as *Nasonia vitripennis* (Walker) (Hymenoptera: Chalcidoidea) (Wylie, 1964), *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae) (Islam, 1994), *Brachymeria lasus* (Walker) (Hymenoptera: Chalcididae) (Husni *et al.*, 2001) and *Diadromus collaris* (Hymenoptera: Ichneumonidae) (Wang & Liu, 2002). Peverieri *et al.* (2013) reported that the development time of the egg parasitoid *Gryon pennsylvanicum* (Hymenoptera: Platygastridae) is longer in older eggs of *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae), and Da Rocha *et al.* (2006) had the same result for *Gryon gallardoi* (Brethes) (Hymenoptera: Scelionidae). Crossman (1925) and Kamay (1976) reported that the development time of *O. kuvanae* was 21 days at 25 °C and 14 days at 30 °C.

In our study, an optimal offspring production rate was obtained using 5-day-old female *O. kuvanae*. The reproductive strategies of parasitoids range from synovigenic to proovigenic. Characteristics of the female such as age may also affect offspring production, particularly in synovigenic parasitoids. If a female parasitoid is synovigenic, such as *O. kuvanae*, she is born with immature eggs (this contrasts with the proovigenic strategy, where adult females have a fixed number of oocytes within their ovarioles) and needs to feed as an adult to sustain egg production. Therefore, increased egg production draws on the energy reserves that could be allocated to extending longevity (Francisco, 2001; Mondy *et al.*, 2006; Jervis *et al.*, 2008). Heimpel *et al.* (1997) reported that synovigenic *Aphytis* females emerge with a fraction of the eggs that can potentially mature during a lifetime. Ueno & Ueno (2007) showed that the rate of oviposition strongly depends on female age; immature eggs are low in number in the earliest stage of the synovigenic female *Itoplectis naranyae* Ashmead (Hymenoptera: Ichneumonidae) and subsequently increase with increasing female age.

Adult nutrition can have important effects on the lifetime reproductive success of female parasitoids (Hagen, 1986; Jervis *et al.*, 1996). Synovigenic parasitoid species can utilize both host hemolymph and non-host foods such as nectar, honeydew and pollen in natural conditions (Jervis *et al.*, 1993, 1996; Heimpel & Collier, 1996; Jervis & Kidd, 1996, 1999; Gilbert & Jervis, 1998). Under laboratory conditions, different adult diets such as different sugars or honey increase longevity and egg maturation (Jervis & Kidd, 1986; Heimpel & Collier, 1996). Carbohydrate sources affect the longevity of adult parasitoids (Jacob & Evans, 1998). A significantly increased mean lifespan for *O. kuvanae* (51 days) may be achieved in the presence of honey, and in the absence of honey, parasitoids died within 3.91 days. Different sugars (solutions of glucose, fructose and sucrose), undiluted bee honey and distilled water have been assessed for adult *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) longevity. The longevity of females fed honey is significantly increased (Perera & Hemachandra, 2014). Tunca *et al.* (2002) evaluated glucose, fructose, sucrose and honey diets for *Chelonus oculator* Panzer (Hymenoptera: Braconidae) and determined that honey is a better diet for parasitoids than other sugars. Similar observations have been made for *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) (Ramos & Cate, 1992) and *Venturia canescens* (Hymenoptera: Ichneumonidae) (Eliopoulos *et al.*, 2005). Crossman (1925) noted a 28–42-day longevity for *O. kuvanae* females, and the same researcher recorded a longevity of up to 130 days for females and 105 days for males under laboratory conditions. On the other hand, Hérard & Mercadier (1980) reported the greatest longevity of female and male parasitoids as 57 and 37 days.

In the current study, a mean of 68.88 ± 3.22 adults developed from the eggs laid by a female reared from *P. ricini*. This value was 68.1 ± 5.0 on *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae) and 32.8 ± 2.8 on *L. dispar* in a previous study (Wang *et al.*, 2013). The mean oviposition and mean post-oviposition periods of the female *O. kuvanae* reared on *P. ricini* were 22.76 ± 1.37 days and 13.64 ± 1.40 days, respectively. According to Wang *et al.* (2013), the mean oviposition period is 25 ± 1.7 days and 21.9 ± 1.9 days for *A. pernyi*-reared *O. kuvanae* females and *L. dispar*-reared *O. kuvanae* females. Several factors may influence offspring production in *O. kuvanae* females. However, more offspring are produced using *P. ricini*, which is an important finding for *L. dispar* biological control programs. Usually, it is more advantageous to use substitute hosts for parasitoids; however, Yang *et al.* (2005) argued that parasitoids are more effective when reared on their original host than on a substitute host. Our results indicate that *P. ricini* is ideal for rearing *O. kuvanae*.

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