

# The Effects of Temperature, Soil Moisture, and Ventilation on the Eggs of the Grasshopper *Romalea guttata*

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## ABSTRACT

*Romalea guttata* (Houttuyn) has a nine-month egg stage in nature. We cultured eggs in the laboratory at 22, 26, 30, and 31.5°C. At low temperatures, hatching was earlier (145 days at 22°C vs. 213 days at 30°C), and survivorship was higher (41 hatchlings/pod at 22°C vs. 0.0 at 31.5°C). Rate of ventilation and flooding of the egg containers did not affect hatching rate. These results show that *R. guttata* eggs survive best at cool temperatures, and also suggest a simple method for culturing the eggs of grasshoppers with long egg diapause.

## INTRODUCTION

Grasshoppers lay their eggs underground in pods (Uvarov 1966; 1977; Stauffer and Whitman 1997). In many species, the eggs develop quickly and hatch in only a few weeks. In contrast, the eggs of many temperate grasshopper species overwinter in the soil for up to nine months before hatching (Uvarov 1966; 1977). During this period, eggs can be influenced by temperature, moisture, and oxygen levels (Uvarov 1966; 1977; Farrow 1975). The eggs are metabolically active as the embryos develop, but oxygen consumption falls during periods when the embryos enter diapause. Although egg dry weight does not vary greatly during development, eggs will absorb or lose water as soil moisture levels rise or fall (Lebouvier et al. 1985). For some species, dramatic changes in soil moisture (such as an intense rainfall) are thought to trigger hatching in nature (Whitman and Orsak 1985). Temperature also influences developmental rates and may serve as a stimulus for hatching (Uvarov 1966; 1977). Extreme soil temperatures or soil moistures are known to kill grasshopper eggs (Uvarov 1966; 1977; Ewer 1977).

Numerous studies have examined the effects of temperature or moisture on egg incubation for species with short incubation periods (Uvarov 1966; 1977; Hewitt 1985). Few studies, however, have examined egg incubation in species with long egg diapause, primarily because of the difficulty in maintaining eggs for long periods (Henry, 1985; Willey 1985; Whitman 1986). In this paper, we report the effects of various soil conditions on egg survival and incubation duration in *Romalea guttata* (Houttuyn), a species with a long egg incubation period. Our results provide information on how to culture long-diapause grasshopper eggs.

The eastern lubber grasshopper, *R. guttata*, inhabits swampy to upland areas throughout the southeastern United States (Rehn and Grant 1961). Eggs are typically laid in late summer and hatch in the spring, and are therefore subjected to wide variations in temperature and moisture levels. To lay, *R. guttata* females dig into the ground with their ovipositor valves to a depth of about 6 cm. They then deposit up to 70 eggs, mixed with a foamy secretion. The foam hardens and forms a protective covering around the eggs, as well as a plug at the top of the hole (Stauffer and Whitman 1997).

## MATERIALS AND METHODS

### Animals

*Romalea guttata* (Houttuyn) were collected near Copeland, FL in June 1996 and brought to Normal, IL, where they were maintained and allowed to mate in the laboratory in a 2 m<sup>3</sup> wire screen cage equipped with two 150 W incandescent flood lights on a 14:10 L:D photoperiod. Grasshoppers were fed Romaine lettuce, oatmeal, and carrot and onion greens.

### Oviposition

Gravid females that were ready to lay usually performed stereotypic probing behaviors with the ovipositor valves (Stauffer and Whitman 1997). Such females were allowed to lay into clear 2000 ml plastic cups filled to 80% capacity with fine inorganic sand (99.9% silica) at 4% moisture level (= 4% of complete soil saturation or 0.8 ml water/100 g sand). One pod was laid per cup. Cups were then sealed with air-tight lids and kept at room temperature (22°C) for 14 days before being assigned to one of the experiments below.

### Temperature Experiment

To determine the effects of temperature on egg development, 39 cups were divided into four treatment groups and cultured in incubators at 22, 26, 30, or 31.5°C. Each sealed cup contained one egg pod, and was opened every 2 weeks for one minute to allow gas exchange. Cups were kept at their assigned temperatures for 270 days. This period was considered long enough to allow complete hatching in those pods which had survived their treatments (most pods hatched in less than 155 days).

### Ventilation and Flooding Experiment

To determine the effects of periodic ventilation and flooding on egg hatch, 40 sealed cups were divided into four treatment groups that differed in the frequency of ventilation -- per week, per 2 weeks, per month, and never opened. During ventilation, the tight-fitting lids were removed for one minute. All cups were maintained for 14 days at room temperature, then for 96 days, in an incubator at 22°C, during which no additional water was added to the cups. When cups were 110 days old, one half of each treatment was flooded; this procedure was designed to simulate an intense rainfall. For flooding, five cups in each treatment were opened and filled to the surface with deionized water, which was allowed to drain out over a period of two days through small holes punched in the bottom of the cups. These "flooded" cups were given screen lids and placed on a laboratory table at room temperature (21-25°C). For all cups, we recorded the number of hatchlings/day. The non-flooded cups remained in the incubator at 22°C.

## RESULTS

### Temperature Experiment

Temperature influenced egg survivorship and length of the egg stage (Table 1). Significantly more pods hatched at lower temperatures than at high temperatures (G-test,  $p < 0.001$ ,  $G = 29.78$ ,  $df = 3$ ), and significantly more young hatched per pod at lower temperatures (Kruskal-Wallis Test,  $p < 0.01$ ;  $\chi^2 = 20.8$ ). At  $31.5^\circ\text{C}$  no pods hatched. Hatching was earlier at lower temperatures (Kruskal-Wallis Test,  $p < 0.001$ ).

### Ventilation and Flooding Experiment

Ventilation rate and early flooding did not affect the number of pods hatching, nor the number of hatchlings per pod (ANOVA,  $p < 0.05$ ) (Table 2). This suggests that *R. guttata* eggs can be successfully cultured at  $22^\circ\text{C}$  in cups that are not ventilated, and that a flooding episode late in egg development is not required to elicit hatching. However, both ventilation rate and flooding significantly influenced the egg incubation period (ANOVA,  $p < 0.05$ ), with flooded eggs and ventilated eggs hatching a few days later on average than unflooded eggs or eggs that were never ventilated.

## DISCUSSION

Our results show that high temperatures negatively influence the eggs of *Romalea guttata*; egg survival decreased and incubation periods increased as temperatures increased. Willey (1985) also noted that higher egg incubation temperatures increased egg mortality and did not shorten egg incubation periods. This is in direct contrast to most larval insects in which higher temperatures generally speed development (Chapman 1971). These data also suggest a fundamental difference in the optimal environmental temperature for grasshopper eggs vs. larvae. Larvae and adults of most grasshopper species prefer, and perform best, at temperatures between  $26$  and  $36^\circ\text{C}$  (Gardiner 1985; Willey 1985; Whitman 1987; Chappell and Whitman 1990). In contrast, the eggs of *R. guttata* appear to perform best at temperatures below  $26^\circ\text{C}$ . In other words, *R. guttata* eggs appear to be adapted to temperatures characteristic of the relatively cool soil environment in which the eggs are found.

The ventilation experiment shows that a lack of ventilation does not harm the developing embryos. Either enough oxygen must exist in the closed containers or sufficient oxygen enters through the sealed lids to allow ample growth and development for the 140-150 days necessary for hatching. In fact, the unopened treatment groups actually had the highest mean number of hatchlings and the shortest average incubation period. The results also show that *R. guttata* eggs do not require a flooding late in their development to elicit hatching, as do some other grasshopper species (Uvarov 1977; Whitman and Orsak 1985).

These results are useful because they suggest that the eggs of some grasshopper species with long egg diapause can be easily cultured in the laboratory using the described methods, which are simple, efficient, inexpensive, and highly amenable to experimental manipulation.

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Table 1. Number of eggs hatching and duration of incubation for *Romalea guttata* eggs cultured at different temperatures.

Culture Temperature (°C)	Pods Tested	Pods Hatched	Hatchlings per viable pod ( $\bar{x} \pm SE$ )	Hatchlings per all pods ( $\bar{x} \pm SE$ )	Median time to hatch (d)
22	10	10	40.8 ± 5.0	40.8 ± 5.0	144.5
26	9	8	15.6 ± 5.3	13.9 ± 5.0	152.5
30	10	3	35.7 ± 4.1	10.7 ± 5.6	213.0
31.5	10	0	0	0	-

Table 2. Number of eggs hatching and duration of incubation for *Romalea guttata* eggs subjected to different ventilation and flooding rates.

Flooded	Ventilation Frequency	Pods Tested	Pods Hatched	Hatchlings per pod ( $\bar{x} \pm SE$ )	Median time to hatch (d)
+	per week	5	5	30.6 ± 9.08	146
+	per 2 weeks	5	5	44.4 ± 9.97	143
+	per 4 weeks	5	5	41.0 ± 9.97	143
+	never	5	5	43.4 ± 1.16	142
-	per week	5	5	37.2 ± 7.07	143
-	per 2 weeks	5	5	42.0 ± 0.72	140
-	per 4 weeks	5	5	34.6 ± 9.88	145
-	never	5	5	49.6 ± 3.98	139