Studies on the embryonic development and embryonic diapause in Arphia conspersa (Scudd.) and Arphia pseudonietana (Thomas) (Orthoptera, Acrididae) and the effects of plant growth hormones on reproduction and diapause
by Russell Allen Jurenka

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Two grasshopper species belonging to the genus Arphia (Orthoptera, Acrididae) were reared in the laboratory to determine their embryonic development and to study the stage at which each overwintered. The effects of plant growth hormones on reproduction and embryonic development also were studied for both species of Arphia.

Arphia conspersa Scudd., collected as adults from a wild population, laid nondiapause eggs from April through July. Embryos of this species developed continuously without a period of diapause and hatched in 40 days at 25°C. In nature fifth instar nymphs overwinter, but when reared in the laboratory with lengthened photoperiods and higher temperatures, these nymphs molted into adults. Most of these adults were sterile and only a few females laid eggs; a high percentage of the eggs laid were nonviable.

Arphia pseudonietana (Thomas), when reared in the laboratory under natural daylengths from August through October, laid diapause eggs that developed to a preblastokinesis stage in 30 days at 25°C. Eggs in diapause were incubated at 5°C for 41 days and upon returning to 25°C the embryos hatched in 21 days. Some embryos resumed development when maintained at 25°C, which suggests that exposure to low temperatures is not always necessary to terminate diapause in this species. Nymphs of A. pseudonietana were reared with long daylengths and fed young grass. The resulting adults laid eggs that exhibited a higher intensity of diapause than eggs collected from females reared under natural daylengths and fed aging grass.

Abscisic acid, a plant growth hormone known to be involved with plant senescence, was fed to A. oonspersa which in nature feeds on young spring grass. Fecundity increased and more embryos entered diapause with this treatment. A. pseudonietana was fed gibberellic acid or kinetin (a synthetic cytokinin), both known to promote plant growth; however, no significant effects on embryonic development or reproduction were observed.

STUDIES ON THE EMBRYONIC DEVELOPMENT AND EMBRYONIC DIAPAUSE IN *ARPHIA CONSPERSA* (SCUDD.) AND *ARPHIA PSEUDONIETANA* (THOMAS) (ORTHOPTERA: ACRIDIDAE) AND THE EFFECTS OF PLANT GROWTH HORMONES ON REPRODUCTION AND DIAPAUSE

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ABSTRACT

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INTRODUCTION

Diapause is a period of suppressed development that allows insects to survive unfavorable conditions in their life-cycles (Andrewartha, 1952). This suppressed development can occur at any of the developmental stages of the insect, from egg through adult. Most species of grasshoppers, which live in temperate climates with a harsh winter, overwinter in the egg in embryonic diapause. However, there are several species in temperate climates that overwinter in the nymphal stage (Brooks, 1958). Diapausing eggs can withstand the low temperatures that occur during the winter months and most species have apparently evolved so that the eggs must be exposed to low temperatures in order to terminate the embryonic diapause.

The amount of time required at low temperatures to terminate diapause in grasshopper embryos and the stage at which embryos enter diapause are variable within a species (Moore, 1948; Matheé, 1951; Church and Salt, 1952; Khalifa, 1957; Slifer, 1958; Slifer and King, 1961; Van Horn, 1966; Visscher, 1971). Further, a single female grasshopper may lay both diapause and nondiapause eggs in the same egg pod (Matheé, 1951; Slifer and King, 1961). Likewise, the crickets, *Gryllus firmus* Scudder and *Gryllus pennsylvanicus* Burmeister, lay diapause and nondiapause eggs in the same pod (Bigelow, 1962; Rakshpal, 1962; Rohani and Walker, 1980; Walker, 1980). The proportion of diapause to nondiapause eggs in *G. firmus* varied from day to day and from female to female (Rohani and Walker, 1980; Walker, 1980). Hunter reported that

adult Australian plague locusts, *Choroicetes terminfera* Walker, laid diapause eggs after emigration to an area of decreasing photoperiod. Adult females started to lay diapause eggs under shorter daylengths within seven days after migration, indicating that diapause induction can be rapid and can occur in fully grown adult females.

These reports suggest that diapause in grasshoppers and crickets is influenced by maternal environmental factors, as has been demonstrated in the silkworm, *Bombyx mori* L. Under long daylengths female *B. mori* release a diapause hormone from the subesophageal ganglion that induces diapause in her embryos, while under shorter daylengths no hormone is released and nondiapause eggs are laid (Fukuda, 1951; Hasegawa, 1951).

One of the environmental factors that could affect embryonic diapause in grasshopper progeny is the maternal diet. It is well documented that both quantitative and qualitative changes in the diet are important in determining reproductive success in grasshoppers (Dadd, 1963, 1973; Engelmann, 1970). Alterations in certain dietary components can lead to increased or decreased fertility. Dietary components can be changed by altering the physiological state of the host plant. Senescent vegetation fed to desert locusts, *Schistocerca gregaria* Forskal, caused a delay in sexual maturation when compared to locusts fed green vegetation. Moreover, when gibberellin (GA_3), a plant growth hormone (PGH) that can retard plant senescence, was added to the diet of senescent leaves, maturation was accelerated and egg laying commenced earlier (Ellis et al., 1965). Visscher et al. (1979) fed western wheatgrass, *Agropyron smithii* Rydb., grown under different

temperatures to the grasshopper *Aulocara elliotti* (Thomas). The production of viable eggs was greater in grasshoppers feeding on grass grown in a cool environment (18°-24°C) than in grasshoppers feeding on grass grown in a warm environment (24°-30°C). When two PGH's, GA_3 and abscisic acid (ABA), were added to the diet of western wheatgrass, a significant decrease in the production of viable eggs occurred (Visscher, 1980). These reports suggest that PGH's may have important effects on grasshopper reproduction.

Plant growth hormones also can affect developmental processes in other arthropods. GA_3 decreased the number of progeny in the mites *Tetranychus telarius* (L.) and *Panonychus ulmi* (Koch) (Eichmeier and Guyer, 1960; Rodriguez and Campbell, 1961), was a sterilant in the cotton leafworm *Spodoptera littoralis* Boisduval (Salama and El-Sharaby, 1972), promoted growth in the aphid *Aphis fabae* Scop. (Scheurer, 1976), aided in the growth of honeybees, *Apis mellifera* L., when fed in an artificial diet (Nation and Robinson, 1966), and affected the pattern of puffs in the giant chromosomes of *Drosophila hydei* Sturtevant (Alonso, 1971). Recently GA_3 also was shown to increase the number of breeding pairs in wild mice, *Mus musculus* L. (Olsen, 1981). ABA, a plant growth inhibiting hormone, has a weak juvenile hormone effect when injected into pupae of *Tenebrio molitor* L. (Eidt and Little, 1970) and partially inhibited vitellogenesis when injected into pupae of *Sarcophaga bullata* Parker (DeMan et al., 1981). A cytokinin (N^6-benzyladenine) was shown to reduce the number of alate offspring in the aphid *Chaetosiphon fragaefolii* (Cockerell) (Schaefers and Montgomery, 1973). Exposure of nymphal and adult *Melanoplus*

sanguinipes (F.) to ethylene decreased the female longevity and increased or decreased the rate of nymphal development depending upon the length of exposure (Chrominski et al., 1982).

Two authors have reported effects from PGH's on the adult diapause of the boll weevil, *Anthonomus grandis* Boheman. Kimbrough (1970) found that kinetin reduced the incidence of adult diapause and Otwell (1971) found that both kinetin and GA_3 reduced the incidence of adult diapause. However, the possible role of PGH's in embryonic diapause in insects has apparently not been studied.

In this study two closely related species of grasshoppers were investigated. *Arphia conspersa* Scudd. lays nondiapause eggs in the spring that hatches during the summer and overwinters as a nymph, while *A. pseudonietana* (Thomas) lay diapause eggs that overwinter and hatch in the spring. These species are sometimes sympatric and both feed on the same plant species (Mulkern et al., 1964). Their diets vary, however, in that adult *A. conspersa* feeds on young spring grasses, whereas adult *A. pseudonietana* feeds on mature late season grass. In experiments presented here both species were reared in the laboratory to establish: 1. The pattern of embryonic development under controlled conditions, 2. Some aspects of embryonic diapause in *A. pseudonietana*, and 3. The factors affecting nymphal overwintering in *A. conspersa*. The influence of PGH's on embryonic diapause was assessed by adding exogenous PGH's to the host plants and feeding them to adult grasshoppers reared under similar environmental conditions.

MATERIALS AND METHODS

Rearing Procedures

The two grasshopper species studied here were collected from native wild populations in Montana. *A. conspersa* was collected in the Story Hills near Bozeman in April of 1980 and 1981. *A. pseudonietana* was collected at Pine Butte, southwest of Bozeman in August, 1980, and near Three Forks in August, 1981. Adults were maintained one pair per cage in plastic cages similar to those described by Visscher (1971). Cages, consisting of screen covered clear plastic cylinders 28 centimeters (cm) high and 21.5 cm in diameter, were placed on paper plates in which a hole was cut to allow insertion of a paper cup, 7 cm in diameter and 8 cm high, filled with a mixture of moist soil and sand for oviposition. Another hole in the paper plate held the food vials which contained Kentucky bluegrass, *Poa pratensis* L., collected from a field site near Bozeman. Adult grasshoppers were reared in an insectary under natural daylengths or long daylengths extended with artificial lights and diurnally fluctuating temperatures ranging from 22°C night to 32°C day.

Incubation of Eggs and Embryonic Staging

Egg pods were collected every day or every other day by sifting the soil in the oviposition cups through a coarse screen. Egg pods then were placed upright in plastic vials, covered with moist sand, and

incubated at 25°C or at 5°C for low temperature exposure. Eggs of both species were removed from the incubator and fixed at regular intervals to determine rate of embryonic development. Eggs were fixed in Bouin's solution at 56°C for one hour, allowed to cool, then rinsed and stored in 70% alcohol. Embryonic development was categorized using the staging criteria established by Van Horn (1966). Staging data were used to determine when eggs should be fixed in experiments in which PGH's were fed to adults.

Feeding Exogenous PGH's

Three PGH's were added at different concentrations to the host grass as it was fed to both species of *Arphia* to observe the effects on reproduction and the incidence of diapause. ABA was fed to adult *A. conspersa* from May to July and GA_3 and kinetin were fed to adult *A. pseudonietana* from July to October. The PGH's were added in solution to the food vial in which cut *P. pratensis* was held upright. The cut ends of the grass were allowed to stand in the hormone solutions for 16-18 hours before being fed to the grasshoppers and distilled water was then used to keep the grass watered. Field grass, collected from the same field site for all experiments, was supplied in this manner every third day. The concentrations of hormones used were as follows: ABA - 0.6, 6.0 and 60.0 mg/l; GA_3 - 6.0 and 18.0 mg/l; kinetin - 10.0 and 20.0 mg/l (mg hormone/l distilled water) (Visscher, 1980, 1982a). ABA and GA_3 were dissolved in 0.4 ml of 95% ethanol and kinetin was dissolved in 0.4 ml of 3M HCL. Both controls and treatments contained the same amounts of solvent. ABA, mixed isomers 90% pure, GA_3, grade

III 90% pure, and kinetin, 6-furfurylaminopurine, were obtained from Sigma Chemical (St. Louis, Missouri).

Egg pods were collected every day from *A. conspersa* and incubated at 25°C for 40 days. The eggs then were removed from the pods, fixed, counted and their viability and morphological stages of development determined. Eggs were fixed at 40 days because embryos of *A. conspersa* will hatch in 40-45 days at 25°C. If ABA were to slow embryonic development or cause the embryos to enter diapause, then it should be apparent by 40 days. Egg pods were collected every other day from *A. pseudonietana* and incubated at 25°C for 90 days. The eggs were then removed from the pod, fixed, counted and their viability and morphological stages of development determined. Embryos of *A. pseudonietana* were observed to slowly develop and hatch after about 100 days when incubated at 25°C. It was assumed that changes in developmental patterns induced by PGH's would be observable by 90 days.

A one-tailed Student's t test and one-way analysis of variance were employed to determine statistical significance between the treatment regimens and the control. Differences were considered significant if a probability value was obtained at or below the 0.05 level.

RESULTS

Embryonic Development and Diapause Experiments

Arphia conspersa

The embryology of *A. conspersa* was determined with eggs obtained from adults collected in May of 1980 which had overwintered as nymphs. The age-stage data for *A. conspersa* are reported in Table I. Embryogenesis occurred without a diapause and 50% of the embryos hatched in 44 days when incubated at 25°C. Embryos hatched between 41 and 58 days after being laid at that temperature.

In nature nymphs of *A. conspersa* hatch from eggs laid in the spring, develop until they reach the fourth or fifth instar and then overwinter. To test whether nymphs in the laboratory would continue to develop without exposure to low temperatures, nymphs were hatched from July 28 to August 19, 1980 from eggs laid in the laboratory. These nymphs were reared 10 per cage under a long photoperiod (15 hours light: 9 hours dark) and warm temperatures (32°C day: 22°C night) in the insectary and fed rye seedlings (*Secale cereale* L.) grown under the long daylength supplemented with wheat bran. Out of 134 nymphs that hatched, 56 survived and became adults. These adults were reared one pair per cage under the same conditions as the nymphs. Eggs, collected every other day, were fixed after 40 days at 25°C unless it appeared that the embryo would hatch. These eggs were incubated at 25°C to monitor their ability to hatch. Table II presents data concerning the

Table I. Morphological stages of embryos of *A. conspersa* after various periods of incubation at 25°C. Numerical data represent the number of embryos fixed at a given age and determined to be at a specific stage.

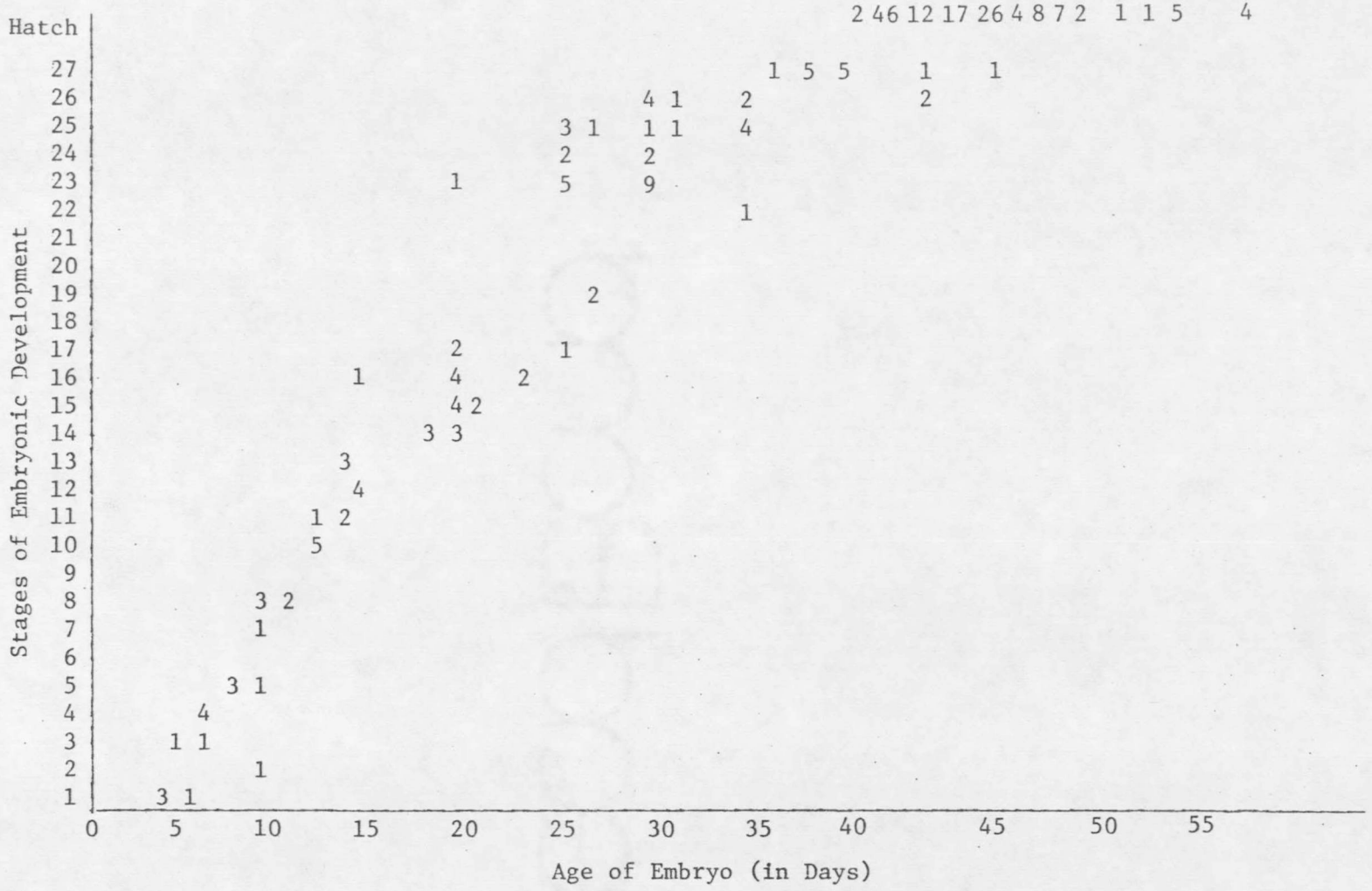

Table II. Reproductive data for adult *A. conspersa* that were not allowed to overwinter as nymphs.

# Adult Pairs	# Pairs Laying Eggs	# Fertile Pairs	# Eggs per Fertile ♀	# Viable Eggs per Fertile ♀	Morphological Stages (# per fertile ♀)		
					19	20-27	Deformed
27	11	6	82.3	9.7	1.5	2.2	6.0

development of these embryos. Only 11 of the 27 adult females laid any eggs, and only six of those 11 females laid fertile eggs. Fertile females laid an average of 82.3 eggs but only 9.7 eggs per female were viable. Six of the 9.7 viable eggs per fertile female contained deformed embryos. Of the remaining 3.7 eggs per female, 1.5 embryos were at Stage 19 and 2.2 embryos were at postblastokinesis stages. None of the embryos that underwent blastokinesis continued development to hatching even when incubated for periods longer than 40 days at 25°C.

Arphia pseudonietana

A. pseudonietana were collected as adults in August of 1980 and eggs collected to determine the length and pattern of embryonic development. These adults were thought to be young because several fifth instar females also were found at the collection site. The age-stage data for embryos of *A. pseudonietana* are depicted in Table III. The data show that embryos of this species develop for about 30 days until they reach Stage 19; then morphological development appears to stop and the embryo enters diapause. This is the stage just prior to blastokinesis, according to the criteria of Van Horn (1966).

Exposure to low temperatures is required to terminate the embryonic diapause of most grasshoppers. Embryos of *A. pseudonietana* that were in diapause after 33 days at 25°C were transferred to 5°C for periods ranging from 23 through 60 days. After low temperature treatments the embryos were replaced at 25°C to observe rates of diapause termination. Diapause was considered terminated if the embryo had undergone blastokinesis. The results of low temperature treatments on