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
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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

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*.

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Abscisic acid, a plant growth hormone known to be involved with plant senescence, was fed to *A. conspersa* 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.

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.

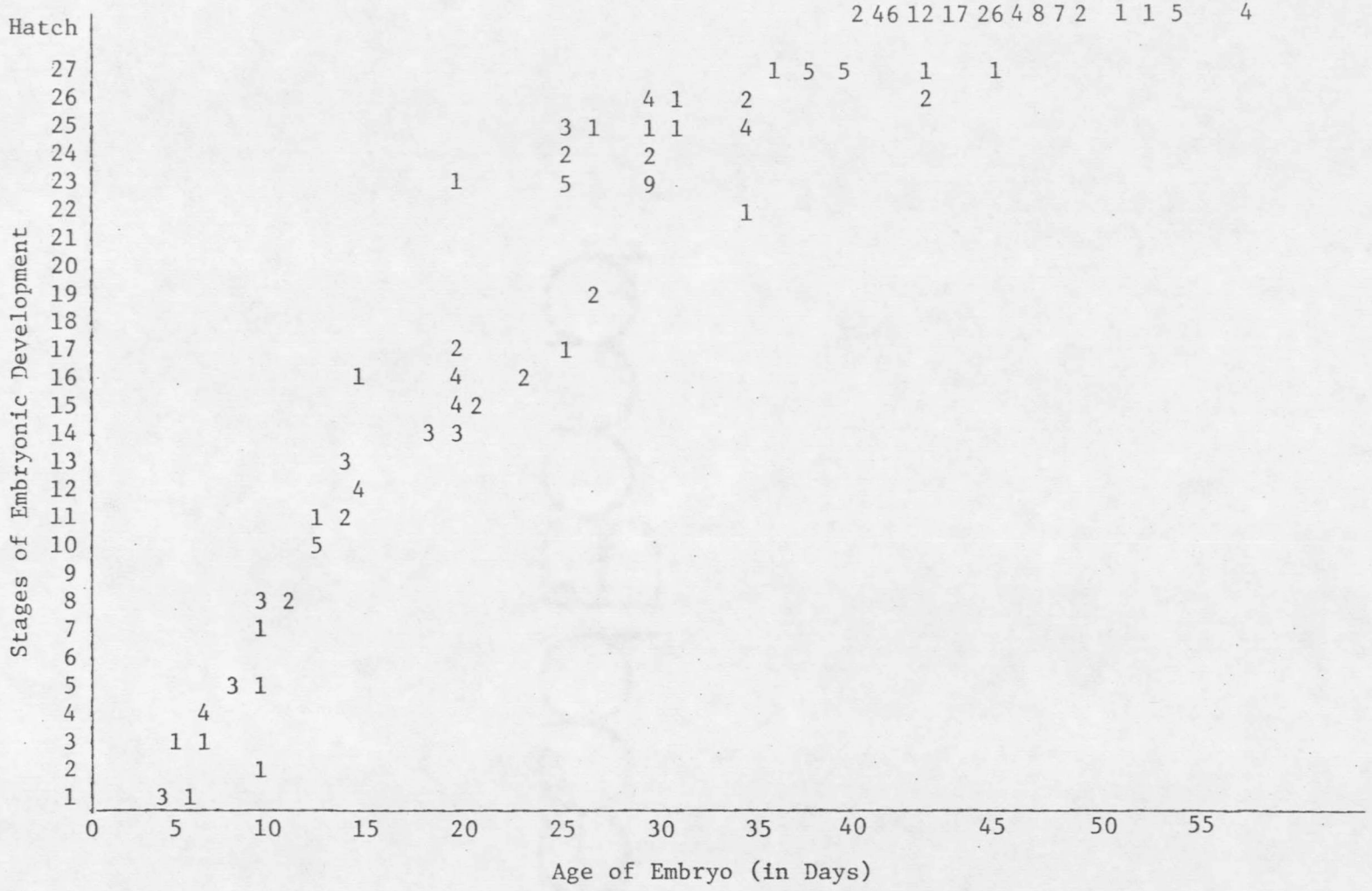


Table II. Reproductive data for adult *A. conspersa* that were not allowed to overwinter as nymphs.

# 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

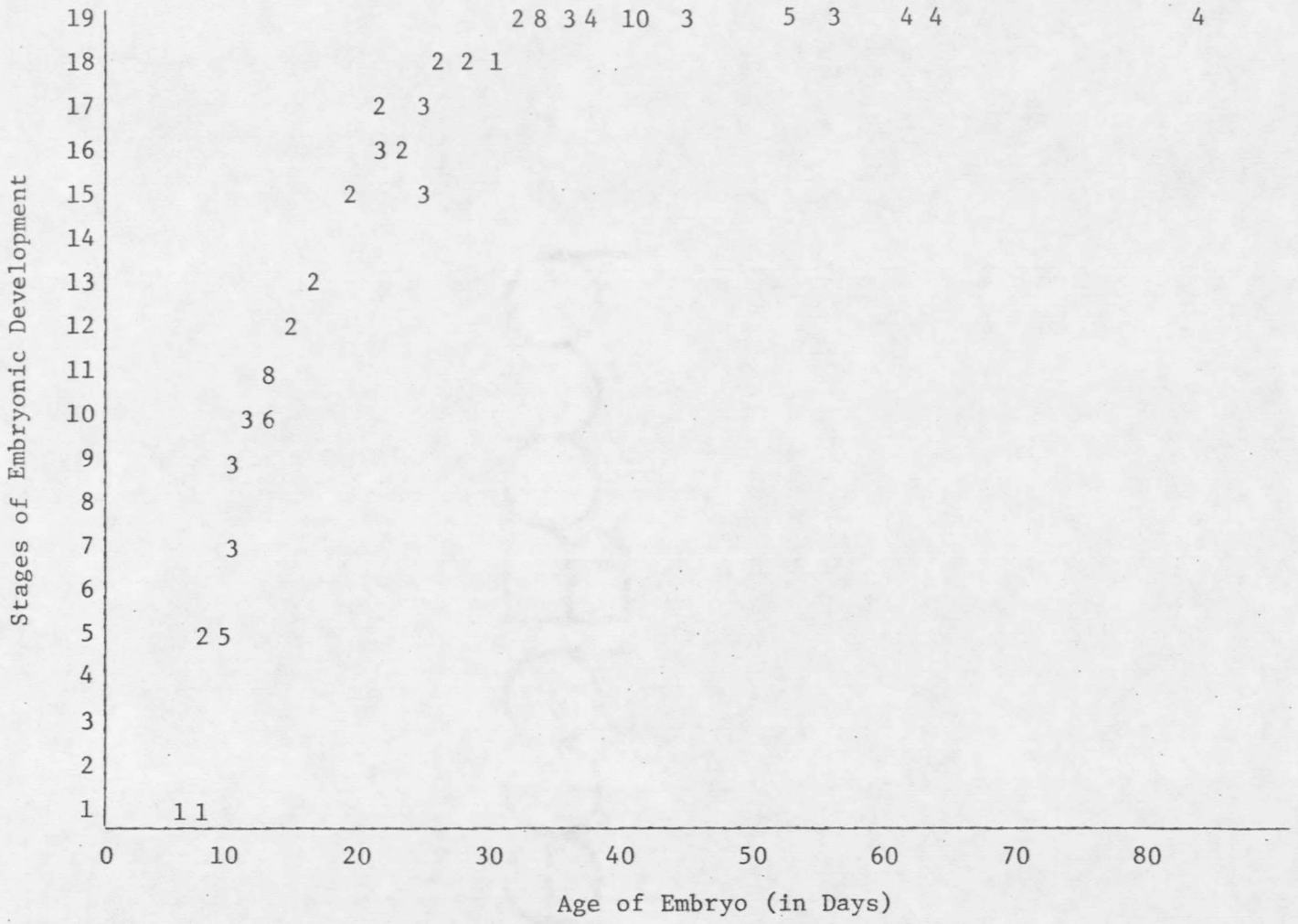
development of these embryos. Only 11 of the 27 adult females laid any eggs, and only six of those 11 females laid fertile eggs. Fertile females laid an average of 82.3 eggs but only 9.7 eggs per female were viable. Six of the 9.7 viable eggs per fertile female contained deformed embryos. Of the remaining 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

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



diapause termination are shown in Table IV. Twenty-three days at 5°C terminated diapause in 25% of the embryos, but none of these embryos hatched. Eighty-four percent of the embryos incubated 30 days at 5°C terminated diapause and 100% of these embryos hatched. Longer exposures to low temperatures terminated diapause in 100% of the viable eggs and almost all of these embryos hatched.

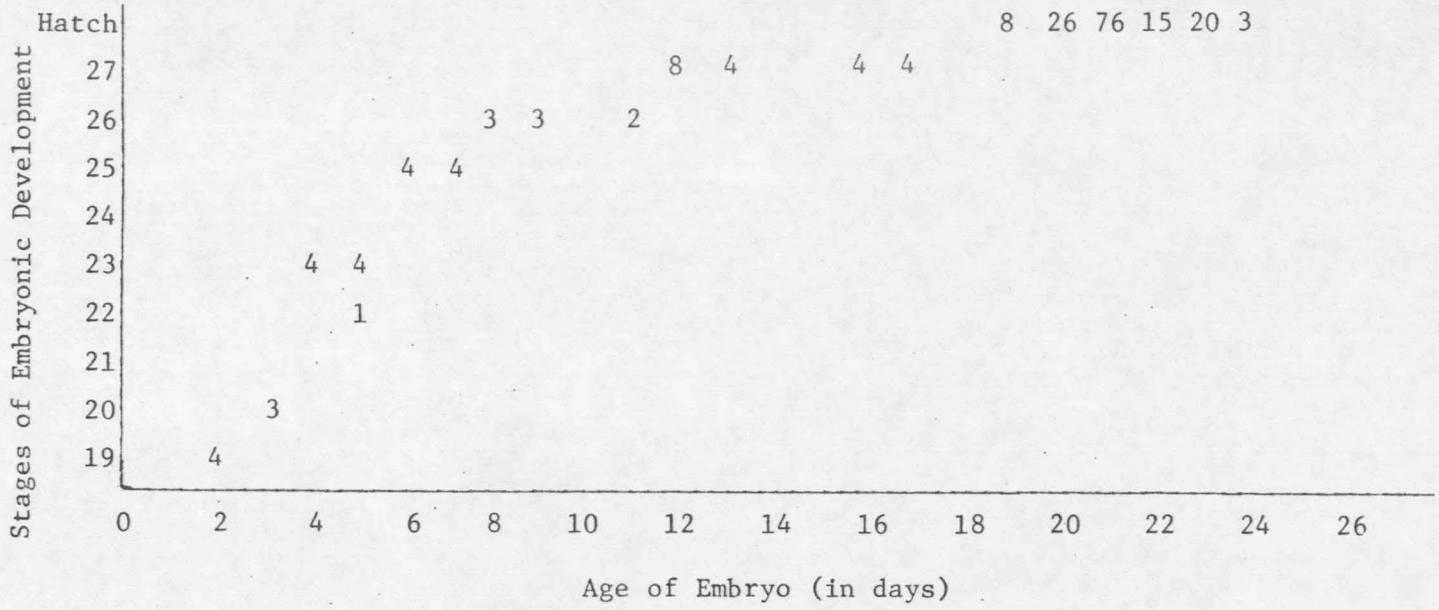
Table IV. Effects of low temperature (5°C) on terminating diapause in embryos of *A. pseudonietana*. Numbers in parentheses refer to the actual number observed terminating diapause or hatching.

Days of Cold Exposure	# Eggs	% Terminating Diapause	% Hatched
23	12	25 (3)	0
30	38	84 (32)	100 (32)
41	151	100 (151)	98 (148)
45	54	100 (54)	100 (54)
60	112	100 (112)	100 (112)

After 41 days at 5°C embryos resumed development when returned to 25°C and 50% hatched in 21 days (Table V). Embryos hatched from 19 to 24 days after removal from 5°C.

Five egg pods of *A. pseudonietana* also were incubated at 25°C for long periods of time to determine whether embryos would remain in diapause. Eggs obtained from field collected females were maintained at 25°C for 120 days after laying. The eggs then were fixed and the stage of embryonic development recorded. The data from these embryos appear in Table VI. Thirty-six nymphs hatched before 120 days when

Table V. Morphological stages of embryos of *A. pseudonietana* after exposure to 5°C for 41 days to terminate diapause. Age of embryo refers to number of days at 25°C after low temperature exposure. Numerical data represent the number of embryos fixed at a given age and determined to be at a specific stage.



the eggs were fixed. Another 11 had developed beyond blastokinesis and 25 remained at Stage 19 120 days after laying. Thus, 65% of the viable eggs after 120 days at 25°C had terminated diapause. Individual pods possessed embryos that were still in diapause (Stage 19) as well as embryos that had terminated diapause. These results show that some embryos do not require prolonged exposure to low temperatures in order to terminate diapause.

Table VI. Staging data for embryos of single egg pods collected from females of *A. pseudonietana* reared in the laboratory under natural decreasing daylengths from August to October, 1981. Eggs were incubated at 25°C for 120 days.

Pod #	Stage 19	Stage 20-27	Hatched	Nonviable	Total # Eggs per Pod
1	2	0	4	14	20
2	0	2	15	2	19
3	4	1	10	1	16
4	11	0	4	1	16
5	8	8	3	2	21
Totals	25	11	36	20	92

All of the eggs described thus far were obtained from field collected grasshoppers that were reared in the insectary from August to October under natural shortening daylengths. To determine whether longer parental photoperiods might induce more embryos to resume development if the embryos were continuously incubated at 25°C, *A. pseudonietana* were maintained under a controlled daylength (15 hours light: 9 hours dark) from time of hatching until death of adults. The

nymphs used in this experiment were obtained from eggs held at 25°C for 33 days, incubated at 5°C for 60 days and then returned to 25°C for hatching. These grasshoppers were fed *P. pratensis* grown in the greenhouse under natural daylengths, rye seedlings grown under long daylengths and wheat bran. It was thought that these rearing techniques would simulate spring rearing conditions even though the experiment was conducted from January to March, 1981.

Three egg pods obtained from adults thus maintained were collected and incubated at 25°C for 119 to 129 days. The eggs were then fixed and their morphological embryonic stages determined. Data obtained with these egg pods are presented in Table VII. Ten embryos were hatched at the end of the incubation period with three more embryos in postblastokinesis stages. However, 28 embryos were still at Stage 19 indicating that they had not terminated diapause. Each pod had at least one embryo that either hatched or underwent blastokinesis. Four egg pods collected from the same adult pairs were also incubated 169 to 176 days at 25°C. Table VIII indicates that seven embryos were found hatched and another seven had undergone blastokinesis before the embryos were fixed. There were 14 embryos still in diapause at the end of the incubation period.

Table IX contains the embryonic staging data comparing eggs collected from females reared under field and laboratory conditions. The highest percentage of embryos terminating diapause were found in those eggs obtained from females reared under field conditions (65%). Only 32% of the embryos collected from females reared under laboratory conditions terminated diapause. Longer incubation of the eggs (169

Table VII. Staging data for embryos of single egg pods collected from females of *A. pseudonietana* reared under a constant photoperiod (15 hours light: 9 hours dark). Eggs were incubated at 25°C for 118 to 129 days.

Pod #	Stage 19	Stage 20-27	Hatched	Nonviable	Age (in Days)
1	12	3	2	2	118
2	10	0	1	3	119
3	6	0	7	0	129
Totals	28	3	10	5	

Table VIII. Staging data for embryos of single egg pods collected from females of *A. pseudonietana* reared under a constant photoperiod (15 hours light: 9 hours dark). Eggs were incubated at 25°C for 169 to 176 days.

Pod #	Stage 19	Stage 20-27	Hatched	Nonviable	Age (in Days)
1	2	3	1	14	169
2	4	2	5	14	169
3	6	1	1	5	174
4	2	1	0	24	176
Totals	14	7	7	57	

to 176 days) collected from females reared under laboratory conditions increased the number terminating diapause to 50%. However, the significance of this percentage is in question due to the large number of nonviable eggs found in those egg pods.

Table IX. Stages and viability of embryos of *A. pseudonietana* collected from adult pairs reared under field or laboratory conditions and incubated for extended periods at 25°C.

	Field[a]	Laboratory[b]	
Days of Incubation at 25°C	120	118-129	169-176
Stage 19	25	28	14
Stages 20-27	11	3	7
Hatched	36	10	7
Nonviable	20	5	57
% Terminating Diapause	65	32	50

[a]Eggs collected from females captured in the field and reared in the laboratory under natural daylengths occurring from August to October, 1981.

[b]Eggs collected from females reared in the laboratory (See text for Rearing Conditions).

Feeding Exogenous PGH's

Arphia conspersa fed ABA

Reproductive data from paired adult *A. conspersa* fed ABA with their host plant are shown in Tables X and XI. Results in Table X were from experiments conducted from late April to late July, 1980. ABA at concentrations of 6 and 60 mg/l increased the total number of eggs laid per female. However, the number of viable eggs laid per female was lowest in the regimen fed ABA at a concentration of 60 mg/l. The mean number of embryos ceasing development at Stage 19 was greater in the groups treated with ABA than in the control. However, no significant differences were found using a one-tailed Student's t test and one-way analysis of variance. The remaining embryos were at Stages 20 through

Table X. Reproductive and staging data for *A. conspersa* fed ABA in 1980. Standard deviations are indicated in parentheses.

Treatment	# Fertile ♀	Mean # Eggs per ♀	Mean # Viable Eggs per ♀	Mean # Stage 19 Embryos per ♀	Mean # Stage 20-27 & Hatched per ♀
Control	7	29.4 (18.7)	16.3 (10.3)	0.4 (0.8)	15.7 (10.4)
ABA - 0.6 mg/l	5	26.4 (12.3)	11.0 (5.6)	1.2 (1.3)	7.0 (5.2)
ABA - 6.0 mg/l	7	39.7 (26.5)	19.0 (18.5)	1.4 (3.0)	16.4 (16.0)
ABA - 60.0 mg/l	7	40.9 (20.9)	9.7 (6.4)	1.1 (2.3)	7.0 (6.1)

Statistical comparisons were between treatment regimens and control (One-tailed Student's t test and one-way analysis of variance).

Table XI. Reproductive and staging data for *A. conspersa* fed ABA in 1981. Standard deviations are indicated in parentheses.

Treatment	# Fertile ♀	Mean # Eggs per ♀	Mean # Viable Eggs per ♀	Mean # Stage 19 Embryos per ♀	Mean # Stage 20-27 & Hatched per ♀
Control	14	54.5 (23.6)	16.9 (19.3)	1.1 (1.8)	11.6 (17.3)
ABA - 6.0 mg/l	12	79.8*(34.7)	33.9*(22.5)	2.0 (2.0)	26.4*(18.3)
ABA - 60.0 mg/l	14	82.1*(46.3)	41.9*(34.1)	4.1*(5.7)	33.1*(28.0)

Statistical comparisons were between treatment regimens and control (One-tailed Student's t test and one-way analysis of variance).

*$p<0.05$.

27 or were hatched by 40 days. A few embryos were deformed and these were not assigned a stage. There were no significant differences found in the number of deformed embryos between the groups.

The results of feeding ABA to paired adult *A. conspersa* from mid-April to mid-August, 1981 are reported in Table XI. This experiment repeated that of the previous year using grasshoppers collected from the same site and fed grass collected from the same field location. Due to the limited number of pairs available at the collection site, the lowest treatment dose (0.6 mg/l) was omitted to increase the number of pairs in the remaining treatments.

Again ABA at both concentrations increased the total number of eggs laid per female compared to the control. Also the number of viable eggs per female was significantly increased in both 6 and 60 mg/l treatments. The number of Stage 19 embryos at 40 days of development was significantly higher in the group treated with ABA at 60 mg/l: 4.1 per female compared to 1.1 per female in the control. The same trend was also noted in 1980 with all regimens treated with ABA yielding more Stage 19 embryos per female. As in 1980, the remaining embryos in 1981 were at Stages 20 through 27, were deformed or had hatched. No significant differences were found between regimens in the number of deformed embryos in 1981.

Significant differences were found between the treatment regimens even though the standard deviations were large. Standard deviations represent the variability within a population. Whenever a wild population of animals is employed, wide variability can be expected.

Arphia pseudonietana fed GA_3 and Kinetin

Table XII contains the reproductive data for adult *A. pseudonietana* fed GA_3 and kinetin from early August through late November, 1981. The control group and groups fed GA_3 at both concentrations laid nearly the same number of eggs per female, while both regimens fed kinetin laid more eggs per female than the control. Those fed the lower dose of kinetin (10 mg/l) laid the most eggs per female. The mean number of viable eggs per female was higher in groups fed kinetin at 10 mg/l and GA_3 at 6 mg/l. However, no significant differences were found between the control and treatment groups in any of the parameters reported. The number of embryos that developed past Stage 19 after 90 days at 25°C varied little between the regimens.

Table XII. Reproductive and staging data for *A. pseudonietana* fed GA_3 and Kinetin in 1981. Standard deviations are indicated in parentheses.

Treatment	# Fertile ♀	Mean # Eggs per ♀	Mean # Viable Eggs per ♀	Mean # Stage 19 Embryos per ♀	Mean # Post-diapause Embryos per ♀
Control	11	200.6 (101.8)	78.3 (53.7)	49.6 (42.9)	20.1 (18.3)
GA_3 - 6 mg/l	10	200.7 (104.0)	85.7 (53.3)	63.0 (43.5)	19.4 (14.4)
GA_3 - 18 mg/l	10	199.9 (66.6)	77.4 (29.5)	48.9 (24.6)	18.9 (17.1)
Kinetin - 10 mg/l	12	233.3 (70.9)	96.3 (34.6)	68.0 (31.0)	19.6 (11.3)
Kinetin - 20 mg/l	13	228.9 (72.1)	70.7 (27.8)	47.7 (23.6)	15.8 (9.5)

Statistical comparisons were between treatment regimens and control (one-tailed Student's t test and one-way analysis of variance).

DISCUSSION

Embryonic Development and Diapause Experiments

Arphia conspersa

In this study field collected *A. conspersa* laid eggs in the insectary from April through June with embryos exhibiting continuous development at 25°C and hatching in about 44 days (Table I). Pickford (1953) also observed continuous development in eggs of this species maintained at 82°F (27.8°C) in the laboratory with hatching occurring after 23 to 27 days of incubation. Raising the temperature 2.8°C appears to hasten development of the embryo considerably. Small temperature changes can affect embryonic development, as shown by data taken from Uvarov (1966, p. 255). Eggs of *Schistocerca*, when incubated at 25°C and 28°C, hatched in about 23 and 17 days respectively, a decrease in 9 days. Therefore, an increase of 3°C could in part account for the shorter development time required for hatching when comparing Pickford's (1953) results with those presented in this study. However, other factors are involved in determining the developmental rate of grasshopper embryos, such as the maternal age and environment and various incubation conditions such as humidity (Shulov, 1970; Visscher, 1971). Whatever the reasons for differences in rate of development, it is apparent that embryos of *A. conspersa* show continuous development when incubated at a constant temperature and do not enter diapause.

Nymphs of *A. conspersa* were prevented from overwintering by artificially extending the photoperiod and rearing at warm temperatures. Most nymphs molted into adults and some adults oviposited. *A. conspersa* can undergo the adult molt during periods of warm winter weather in Colorado (Halliburton and Alexander, 1964). However, *A. conspersa* nymphs reared in a Canadian laboratory developed normally until the fourth instar at which time molting ceased (Pickford, 1953). Pickford (1953) unfortunately did not describe the rearing conditions of the nymphs except to say that they were reared in the laboratory. In this study conditions required for the adult ecdysis of *A. conspersa* were attained without exposing the nymphs to low temperatures.

Adult *A. conspersa* obtained by preventing nymphs from overwintering were largely sterile and their eggs were mostly nonviable, and the majority of viable eggs contained deformed embryos (Table II). Whatever factors required by the adult to produce significant numbers of viable eggs were apparently absent. The photoperiod appeared to be sufficient to support development. However, the duration of the light period was not altered from 15 hours, the approximate daylength occurring in late July at Bozeman (U.S. Nautical Almanac Office, 1981). Perhaps the diet of rye seedlings and wheat bran lacked factors required for successful reproduction. Rye is not reported to be a host plant of *A. conspersa* in nature, and indeed, there are numerous reports demonstrating unfavorable effects of unsuitable host plants on grasshopper reproduction (Pfadt, 1949; Smith et al., 1952; Barnes, 1955; Pickford, 1958, 1962; McCaffery, 1975). Nymphs also may require exposure to low

temperatures for successful reproduction to occur. Further research in this area must be conducted to draw proper conclusions.

Arphia pseudonietana

Field collected adults of *A. pseudonietana* when reared in the insectary laid their eggs from August through October. The embryos developed for about 29 days at 25°C and entered diapause at Stage 19 (Table III). Many grasshopper species have been reported to enter diapause at this pre-blastokinesis stage (Uvarov, 1966). Exposure to low temperatures for at least 41 days will terminate diapause (Table IV).

Eggs of *A. pseudonietana* maintained at 25°C entered diapause at Stage 19 but after an extended period of time, underwent blastokinesis and continued development (Table VI). This indicates that some embryos do not require exposure to low temperatures in order to terminate diapause. After 120 days at 25°C embryos within a single pod were either still arrested at Stage 19 or had continued development and hatched. Because of high mortality rates encountered, it is not certain whether all embryos would eventually hatch if incubated at 25°C for more than 120 days. Church and Salt (1952) observed that a certain percentage of the eggs of *Melanoplus bivittatus* (Say) terminated diapause and hatched when maintained at 25°C for prolonged periods of time. Bigelow (1962) and Rakshpal (1962) also found this to be true in *Gryllus pennsylvanicus* Burmeister, a species of cricket from North America. These results and those reported here suggest that in at least a few eggs incubated at 25°C time alone is sufficient to terminate diapause.

However, exposure to 5°C may provide termination of diapause in 100% of the embryos. It is not known whether low temperature exposure of limited duration (41 days at 5°C) and constant temperature exposure (25°C) are acting upon the same physiological process which terminates diapause in the embryo.

When nymphs of *A. pseudonietana* were reared under long daylengths and fed young grass, the subsequent adults laid eggs that followed a pattern of development similar to that of eggs collected from females reared under decreasing photoperiods and fed aging grass (Contrast Field and Laboratory columns in Table IX). Females from both rearing conditions laid eggs that either terminated diapause and continued development or were still in diapause after prolonged periods at 25°C. However, it appears that a stronger diapause occurred in those eggs laid by females reared under laboratory conditions. This is evident by comparing the percentage of embryos terminating diapause in both rearing conditions. In the eggs collected from field reared females, 65% terminated diapause by 120 days whereas, only 32% of the embryos terminated diapause in eggs collected from females reared under laboratory conditions. Moreover, longer incubations of eggs from laboratory reared females (169-176 days) did not increase the percent terminating diapause to the level observed in eggs from females reared under field conditions.

Differences in rearing conditions between the two regimens include both photoperiod and diet. Adults reared under field conditions were exposed to naturally decreasing daylengths from August to October (14 hours 45 minutes to 10 hours 15 minutes) while those from laboratory

conditions were reared under a constant photoperiod (15 hours light: 9 hours dark). Krysan et al. (1977) found a greater intensity of embryonic diapause in a southern subtropical species of Chrysomelidae, *Diabrotica virgifera* (LeConte), than those found in a northern temperate climate. *Telegryllus emma* Walker, a species of cricket found in Japan, also showed an increasing intensity of embryonic diapause in southern latitudes when compared to northern latitudes (Masaki, 1978). Although these authors do not indicate that a longer photoperiod in southern climates produced an increase in diapause intensity, their observations do suggest a correlation. Perhaps the relatively long photoperiod could cause females to lay eggs with a more intense embryonic diapause.

The effects of diet on the intensity of embryonic diapause has apparently not been studied. In this study the young grass fed to grasshoppers reared under laboratory conditions would likely be correlated with an increasing photoperiod in nature. Diet and photoperiod could be acting together to increase the intensity of diapause. The separate effect of diet on diapause was not assessed in this study. However, the results of feeding exogenous plant growth stimulating hormones (GA_3 and kinetin) to *A. pseudonietana* suggested that these hormones do not have an effect on whether the embryos enter diapause.

Changes in environmental conditions of paternal adults did not change the incidence of diapause, but the intensity of diapause may be altered. Embryos within a single pod possessed varying diapause intensities. Embryos maintained at 25°C, which hatched within 120 days, would have a weak diapause, while those still at Stage 19 after

176 days seemed to possess a stronger diapause. A paternal component may be present, judging from the various developmental rates of the embryos which were held at 25°C. Walker (1980) also found variations in the rate of embryonic development between diapause and "fast-developing" eggs in *Gryllus firmus*. He attributes mixed oviposition of "fast-developing" and diapause eggs as a function of the female's environment and of the embryonic environment. Hartly and Warne (1972) also attributed maternal factors to whether eggs averted an initial diapause in tettigonids. The physiological bases which determine the developmental rate and diapause intensity in grasshopper embryos are unknown.

Feeding Exogenous PGH's

Arphia conspersa fed ABA

In the spring the adults of *A. conspersa* in nature feeds on young growing grass, and in the absence of stress this grass would contain a low level of ABA. If ABA, recognized as a plant growth inhibiting hormone, was increased in the host grass, it was postulated the female would lay fewer eggs and fewer viable eggs. Indeed, Visscher (1980) observed that fewer eggs and fewer viable eggs were laid when exogenous ABA was fed to the grasshopper *Aulocara elliotti*. However, in this study ABA increased both the number of eggs and the number of viable eggs laid per female when fed to *A. conspersa*.

The contrasting results between Visscher (1980) and those reported here are interesting because the same concentrations of ABA were utilized and feeding was conducted in a similar manner. However,

Visscher (1980) exogenously added ABA to *Agropyron smithii*, a perenial wheat grass that underwent natural aging during the summer as it was fed to *A. elliotti*, while in this study ABA was added to *P. pratensis*, also a perennial grass but that was fed in May and June when it was young and actively growing. When ABA is added to young growing grass, perhaps a synergistic effect could occur which caused grasshoppers to lay more eggs. A similar effect has been observed with other insect species.

Scheurer (1976) fed leaf discs containing ABA to the aphid *Aphis fabae* and observed increased fecundity. Gaudet (1978) observed that ABA caused the production of alate progeny in the aphid *Rhopalsiphum padi* (L.). However, vitellogenesis was inhibited when ABA was injected into adult fleshflies *Sarcophaga bullata* (DeMan et al., 1981). Visscher (1982b) reported variable results when ABA was added exogenously to the host plant or fed with an artificial diet to a variety of insects. From these studies it appears that ABA can elicit both stimulatory and inhibitory responses in insects depending upon the species utilized and the dietary test system involved. Research with ABA in plants has also shown both stimulating and inhibitory responses depending upon the plant and tissue involved (Letham et al., 1978). The physiological basis for these contrasting effects of ABA in plants or in insects are unknown (Visscher, 1982b).

ABA was fed to *A. conspersa* not only to observe effects on reproduction but also to try and induce females to lay diapause eggs. Females fed ABA did lay more eggs containing Stage 19 embryos after 40 days of incubation at 25°C (Tables X and XI). This is the same stage

at which embryos of *A. pseudonietana* enter diapause. The embryos of *A. conspersa* can be considered to be in diapause, because morphological development would have been arrested for about 15 days. Normal development to Stage 19 at 25°C takes approximately 25 days so the remaining 15 days were spent in arrested development. Another explanation is development was so retarded that 40 days was required for the embryo to reach Stage 19. This is unlikely, however, because the embryos appeared morphologically normal with no abnormalities that sometimes accompany retarded development.

In this experiment embryos of *A. conspersa* were fixed at 40 days because longer incubation would normally result in hatching. If a longer incubation period had been chosen, diapause might have been terminated at 25°C, as it was in *A. pseudonietana*. Embryos which would have undergone blastokinesis would be considered to be non-diapause; if a diapause had ensued, it would not have been observed.

Both the females fed ABA and the control females laid diapause eggs, suggesting that embryonic diapause can occur in nature. The ability to lay diapause eggs has survival advantage in the unpredictable climate occurring in temperate latitudes. If temperatures were too low to permit completion of embryonic development during the summer, the embryo could overwinter with the nymph hatching the following spring. In this regard, Pickford (1953) observed overwintering eggs in wild populations of *A. conspersa* in central Saskatchewan, Canada, indicating that eggs of *A. conspersa* can sometimes overwinter in nature.

The reasons why ABA caused the production of more diapause eggs in *A. conspersa* are unclear. ABA has been implicated as an inhibitor

involved in the dormancy of seeds (Letham et al., 1978). It may be possible that ABA could act as an inhibitor in the embryonic diapause of *A. conspersa*. However, it is not known whether ABA acts directly or indirectly on the adult grasshopper, or directly on the embryo. Somehow ABA stimulates the adult female grasshopper to produce more eggs, but it also may act on the embryo causing it to enter diapause. Further research in this area must be conducted to elucidate the mechanism or mechanisms by which ABA affects the reproduction or development of *A. conspersa*.

Arphia pseudonietana fed GA_3 and Kinetin

When adult *A. pseudonietana* were fed GA_3 or kinetin, no significant differences were found in the number of eggs laid per female or in the number of embryos that terminated diapause in any of the treatments (Table XII). However, the lower dose of kinetin gave the largest number of eggs and viable eggs laid per female. These observations suggest that the concentrations of PGH's utilized may have been too high. Lower concentrations might have produced different results. Results of this study suggest that the plant hormones GA_3 and kinetin do not affect the embryonic diapause of *A. pseudonietana* at the concentrations utilized.

SUMMARY

A. conspersa laid nondiapause eggs in the spring that hatched in approximately 40 days at 25°C. In nature fifth instar nymphs overwinter; however, when reared in the laboratory with artificially lengthened photoperiod and warm temperatures, these nymphs molted into adults. The resulting adults were mostly sterile but a few females laid eggs and the majority of these eggs were nonviable. These results indicate that developmental conditions were met for nymphs to molt into adults but not for the adults to lay fertile eggs.

A. pseudonietana laid diapause eggs in the fall. The embryos developed for 30 days at 25°C and entered diapause at a stage just prior to blastokinesis. When diapause eggs were incubated for 41 days at 5°C, the embryos terminated diapause and hatched in 21 days after returning to 25°C. When diapause eggs were maintained at 25°C for long periods, some embryos terminated diapause while others remained in diapause. This indicates that exposure to low temperature is not necessary to terminate diapause in some embryos. This also suggests that the intensity of the diapause can vary between embryos. When nymphs and adults of *A. pseudonietana* were reared under a constant photoperiod (15 hours light: 9 hours dark), females laid eggs that possessed a stronger diapause than that observed in eggs laid by females reared under natural daylengths. The relatively longer photoperiod apparently induced a stronger diapause.

When ABA was fed to *A. conspersa*, the production of eggs increased and more embryos were observed to be in diapause after 40 days at 25°C. There were no significant effects on reproduction or embryonic development when GA_3 and kinetin were fed to *A. pseudonietana*.

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