Factors influencing the determination of wing formation in the aphid, Rhopalosiphum padi (L.) (Homoptera: Aphidae)
by Michelle Diane Hardisty Gaudet

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Abstract:
The determination of wing form in the aphid Rhopalosiphum padi (L.) was examined with respect to photoperiods and- plant hormones (GA- = gibberellic acid and ABA = abscisic acid) and the underlying physiological mechanisms were considered.

Virginoparae of R_. padi were reared under two photoperiodic regimens. One group was reared under increasing photoperiods, while the other group was reared under decreasing photoperiods. When the number of alate and apterous offspring produced were compared between regimens it was found that aphids reared under increasing photoperiods produced more apterous progeny than those reared under decreasing photoperiods.

Two plant hormones were tested for possible effects upon wing dimorphism in R. padi and a significant treatment effect (P = 0.01) was received at the F10 generation in the 16-hour photoperiodic regimen. Whether these plant hormones influence wing dimorphism in the aphid via changes in the physiology of the host plant or directly on the physiology of the aphid is unknown.

The volume of the corpus allatum in R. padi was measured as a parameter of its activity. Aphid mothers that produced mostly apterous progeny had a larger corpora allata (P = 0.035) than those mothers that produced predominantly alate offspring. The data obtained confirm the studies of White (1971). It is proposed from these results that corpus allatum activity and high titers of JH are associated with the production of apterous offspring.

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ABSTRACT

The determination of wing form in the aphid Rhopalosiphum padi (L.) was examined with respect to photoperiods and plant hormones (GA_3 = gibberellic acid and ABA = abscisic acid) and the underlying physiological mechanisms were considered.

Virginoparae of R. padi were reared under two photoperiodic regimens. One group was reared under increasing photoperiods, while the other group was reared under decreasing photoperiods. When the number of alate and apterous offspring produced were compared between regimens it was found that aphids reared under increasing photoperiods produced more apterous progeny than those reared under decreasing photoperiods.

Two plant hormones were tested for possible effects upon wing dimorphism in R. padi and a significant treatment effect ($P = 0.01$) was received at the F_{10} generation in the 16-hour photoperiodic regimen. Whether these plant hormones influence wing dimorphism in the aphid via changes in the physiology of the host plant or directly on the physiology of the aphid is unknown.

The volume of the corpus allatum in R. padi was measured as a parameter of its activity. Aphid mothers that produced mostly apterous progeny had a larger corpora allata ($P = 0.035$) than those mothers that produced predominantly alate offspring. The data obtained confirm the studies of White (1971). It is proposed from these results that corpus allatum activity and high titers of JH are associated with the production of apterous offspring.

INTRODUCTION

The aphid Rhopalosiphum padi (L.) is an important agricultural pest in Montana, not only because it feeds upon cereal crops, but because it is a vector of the RPV isolate of Barley Yellow Dwarf Virus (Harris and Maramorosch, 1977). The development of effective control of this pest requires a thorough understanding of its ontogenesis, in particular the factors which determine the production of winged forms.

Several environmental factors, including photoperiod, temperature, humidity, nutrition and crowding can influence alary polymorphism in aphids (Lees. 1966). These environmental regulators are thought to act through the neuroendocrine system, and considerable research has been directed towards describing the developmental processes and understanding the physiological mechanisms involved (Johnson and Birks, 1960; Johnson, 1966; Lees, 1966; White, 1965; 1968; 1971; 1972; White and Gregory, 1972).

Wing dimorphism in R. padi has been examined in terms of crowding (Dixon and Glen, 1971), nutritional status (Noda, 1956; Dixon and Glen, 1971) and the critical time of wing development (Noda, 1958). Poor nutrition and crowding stimulated the production of alates, with crowding being most effective late in the first instar (21 hours after birth).

The present investigation was undertaken to evaluate the role of photoperiod as a direct environmental stimulus to wing development and

to test the possibility that environmental factors could act indirectly to stimulate wing formation through changes in hormone titers of the host plant. Corpus allatum activity was examined in conjunction with the production of alate versus apterous offspring.

LITERATURE REVIEW

Photoperiod

The effect of photoperiod on form production and wing dimorphism was reviewed by Lees in 1966. He concluded that in heteroecious species of aphids, longer photoperiods cause a higher production of alate progeny. Current theories concerning this photoperiodic response have been complicated by the disagreement among authors as to whether the photoperiod acts directly on the aphid to produce the response (Lees, 1966) or indirectly through nutritional changes in the aphids' host plant (Johnson, 1966; Sutherland, 1969; Harrewijn, 1972; 1976a; 1976b).

Plant Hormones

The importance of parental nutrition from the host plant on alary polymorphism in aphids has been well documented in the literature. Myzus persicae Sulz. was found to produce more alates when fed on plants in flower than when fed on plants in the young or post flowering stages (Pintera, 1957). Apterae of Aphis craccivora Koch. reared on mature bean foliage produced more alate offspring than those maintained on germinating seedlings (Johnson and Birks, 1960; Johnson, 1965). With aphids reared on young foliage, especially on seedlings, wing dimorphism was shown to be directed towards the apterous course (Schaefers and Judge, 1971; Harrewijn, 1972; 1973).

Much emphasis has been placed on the host plant fluctuation of various nutrients such as amino acids, sugars, sterols and ions in response to environmental conditions (photoperiod and temperature) previously associated with wing dimorphism (Dadd, 1968; Mittler, 1972; Harrewijn, 1972; 1973; 1976a; 1976b; White, 1972; Lechstein and Llewellyn, 1973). Limited consideration, however, has been given to the role of plant hormones in the phenomenon of wing dimorphism. Cytokinin (N^6 Benzyladenine) was found to cause the production of fewer alates in the aphid Chaetosiphon fragaefolii (Cockrell) (Schaefers and Montgomery, 1973). Gibberellic acid (GA_3) and abscisic acid were found to reduce the time needed for development in Aphis fabae Scop. (Schreurer, 1976).

Corpus Allatum

The aphid corpus allatum gland was first observed by Nubert in 1913 during investigation on the nervous system of Rhynchotes (Cazal, P. 1948). Pflugfelder (1936) described the corpus allatum and the two corpora cardiaca of Pemphigus bursarius (L.) and found the retrocerebral organs in this aphid were well formed and distinct. Cazal (1948) studied three species of aphids, Macrosiphum rosae Réaumer, Aphis sp. and Pemphigus sp. with respect to their retrocerebral organs and found that these were very distinct in Pemphigus, but in the other two the corpora paracardiaca (corpora cardiaca) were difficult to determine.

He noted, however, that the sympathetic nervous system was similar to that described for hemipterans.

Cazal's work several histological studies concerning neurosecretion in aphids have been published (Johnson, 1962; 1963; Gabriel, 1965; Bowers and Johnson, 1966). Other researchers have described the stomatogastric nervous system of the green peach aphid (Takaoka, 1969) the greenbug (Saxena and Chada, 1971) and Macrosiphum euphobiae (Thomas) (Paré et al., 1974).

The physiological mechanisms underlying the control of wing dimorphism in aphids are still a subject of controversy (White, 1971). Much information has been obtained by histological studies of the developmental processes involved in the production of different forms. The findings of Johnson and Birks (1960) suggested that all aphids begin development as presumptive alatae and that apterae and intermediates are forms which have been diverted from the alate course during development. Research undertaken by White (1971) confirmed these conclusions and contributed the evidence that the regression of wingbuds in apterous forms bore a close relationship to the activity of the corpus allatum in the parents and larvae. The extent of loss of wingbuds in embryos appeared to depend upon maternal corpus allatum activity during the time the embryo was developing. In postnatal determination of apterae, which can occur up to 48 hours after birth (Kawada, 1965), higher corpus allatum activity of the individual aphid appeared to be

the controlling factor. Also Rhopalosiphum padi (L.) has been shown to produce alates by crowding nymphs at twenty-one hours after birth (Noda, 1958).

MATERIALS AND METHODS

Photoperiod

Virginoparae of *Rhopalosiphum padi* (L.) were collected on *Triticum aestivum* (L.) of an unknown cultivar grown in a greenhouse at Montana State University on July 6, 1977 and transferred to a greenhouse insectary onto the spring wheat cultivar 'Norana', *T. aestivum*. Apterous aphids and their host plants were then separated into two photoperiod regimens. One group was reared in a Percival controlled environment chamber at photoperiods increasing from 14 hours daylength on July 6, 1977 to 16 hours daylength beginning on August 6, 1977 and ending September 2, 1977. The other group was reared in a controlled environment room at ambient photoperiods decreasing from 15 hours and 31 minutes on July 6, 1977 to 13 hours and 13 minutes of daylength by September 2, 1977. Temperatures in both environments fluctuated diurnally (18-24.5 C). Aphids were reared on single wheat plants grown in aluminum cake pans (22.9 cm in diameter X 8.9 cm deep) placed under cloth (chiffon) covered, clear plastic cylinders (27.9 high X 21.6 cm in diameter). To avoid crowding, no more than 40 aphids were allowed to develop within a single cage. Aphids were transferred to new cages and handled using techniques described by H. F. Van Emden (1972).

Plant Hormones

Virginoparae of the aphid *R. padi* were reared according to methods described in the photoperiod section. F_6 apterous adults were taken

from culture colonies maintained in the two photoperiodic regimens and transferred (September 9, 1977) one per cage onto the spring wheat cultivar 'Norana'; T. aestivum (seeded August 23, 1977). Twelve cages of aphids were maintained at the 16-hour photophase in a Percival controlled environment chamber, while twelve additional cages were maintained in a second controlled environment chamber at ambient photoperiod decreasing from 12 hours 58 minutes to 8 hours 50 minutes daylength (U.S. Nautical Almanac Office, 1977). Temperatures in both environments fluctuated diurnally from 18-24.5 C, as in the photoperiod experiment.

Six treatments, with two replicates each, were administered to aphids in each photoperiod regimen, beginning upon the observation of the first F_7 nymphs (September 10-September 12, 1977). Ten ml of treatment solution were applied per cage on alternate days, with six ml sprayed onto the aphids and the leaves of the plant, while the remaining four were watered to the base of the plant. On non-treatment days, the plants were given distilled water. Hormone solutions (gibberellic acid and abscisic acid)[1] prepared by dissolving the quantity of the solid hormone needed in 20 ml 95% ETOH and diluting this solution with one liter of distilled water, were administered at two concentrations

1. The gibberellic acid used by the author is referred to as GA_3 if the type of gibberellin is not specified by other authors referred to in this text, it will be designated as GA. ABA refers to abscisic acid.

(6 mg / L dist H_2O & 60 mg / L dist H_2O) (Visscher, 1977, unpublished data).[1]

To avoid crowding, the number of offspring produced was limited so that total number of aphids in the cage never exceeded ninety, with the average being forty. The youngest nymph in the cage, regardless of form, was chosen to be the parent of the next generation. The wing form of the offspring was determined at the adult stage. Results were analyzed statistically beginning with the F_{10} generation, allowing three generations to insure that the results expressed would be those of the treatments tested rather than those of previous rearing conditions (Noda, 1958; Lees, 1966).

Measurement of the Corpus Allatum

Thirteen adult aphids which produced mainly apterous offspring and thirteen adults which produced mostly alate offspring were taken from the two experiments described. These aphids were fixed in alcoholic Bouin's solution containing 0.5% trichloroacatic acid and double embedded in methyl-benzoate-celloidin solution followed by paraffin. Aphids were sectioned transversely at 8μ and stained following the procedure for Ewen's Aldehyde-fuchsin (Barbosa, 1974) (see appendix for the adaptation of this procedure for use with aphid tissues). The length and width of the corpus allatum were measured in a section at

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its maximum representation using a screw-type eyepiece micrometer at 800x and the approximate volume of the gland was calculated assuming it to be an ellipsoid having equal minor axes as described by White (1965) and Burrington (1973).

In this paper, the corpus allatum activity (by calculation of gland volume) of mothers that produced predominantly apterous offspring is compared with mothers that produced mostly alate offspring. The form of the offspring was judged upon rearing to adulthood, rather than through estimations of wingbud degeneration in the embryo as in the experiments performed by White (1971) and would be a more accurate assessment of the final form of the aphid. Since postnatal determination of form has been demonstrated in R. padi, a correlation obtained between the corpus allatum activity of the mother and form determination of the offspring would suggest that prenatal determination was the predominant pattern in this species.

RESULTS

Photoperiod

The numbers of alate and apterous offspring produced in each of the F_1 through to the F_5 generations at the two photoperiods are shown in Table I. The number of alate and apterous adults produced in each of these two photoperiod regimens are presented in Table II. A total of 571 aphids were reared in the two regimens with 282 reared under ambient photoperiods and 289 reared at the 14-16 hour photoperiods. Sixty alate and 222 apterous adults were produced under ambient photoperiods, while 25 alates and 264 apterous adults were produced in the 14-16 hour photoperiods. Comparison of these results by Chi-Square analysis revealed significant differences between regimens (X^2 = 16.9756, P = 0.00002).

A significant treatment effect was observed between the two photoperiod regimens. More apterous individuals were obtained under increasing photoperiods than were obtained under decreasing photoperiods. This is in accordance with the results obtained by MacGillivry and Anderson in 1964; Johnson in 1965; and White in 1946, with Macrosiphum euphorbiae, Aphis craccivora, and Macrosiphoniella sanborni respectively as cited by Lees (1966). The results suggest that Rhopalosiphum padi (Linn.) responds similarly to other species of heteroecious aphids with respect to photoperiod in determination of the production of alate versus apterous offspring, i.e., increasing photoperiods cause

TABLE I. THE NUMBER OF ALATE and APTEROUS OFFSPRING IN EACH OF THE F_1 THROUGH TO THE F_5 GENERATIONS AT TWO PHOTOPERIODS.

	F_1 Generation		F_2 Generation		F_3 Generation		F_4 Generation		F_5 Generation	
	# Alate	# Apterous	# Alate	# Apterous	# Alate	# Apterous	# Alate	# Apterous	# Alate	# Apterous
*Ambient Photoperiods	0	2	21	21	11	62	27	97	1	40
14-16 hr. Photoperiods	4	15	7	42	0	58	0	70	14	79

TABLE II. TOTAL NUMBER OF ALATE AND APTEROUS ADULTS PRODUCED IN EACH OF THE TWO PHOTOPERIODIC REGIMENS.

	# of Alate Adults	# of Apterous Adults	Total # of Aphids
*Ambient Photoperiod	60	222	282
14-16 hr. Photoperiod	25	264	289
Total # of aphids	85	486	571

*Ambient photoperiod was 15 hrs and 31 minutes of daylength at the beginning of the study and 13 hrs and 13 minutes at the end of the study.

the production of apterous offspring while decreasing photoperiods initiate the production of alate offspring.

Plant Hormones

The results of treatments conducted over 11 generations on wing dimorphism in the 16 hour photoperiod regimen are shown in Table III. No data are available for the tap water controls after the F_{11} generation due to deaths of the adults chosen to be the parents of the F_{10} and F_{11} generations in cages #32 and #38.

Table IV depicts the influence of treatments conducted over eight generations on wing dimorphism in the ambient photoperiod regimen. Treatments in this regimen facilitated the growth of powdery mildew on the plants, resulting in the death of some plants and the aphids thereon and no data were obtained in these.

In the above Tables (III & IV) the form of the mother is depicted as A, B, or C meaning respectively, alate, apterous or both alate and apterous. To estimate the age of the mother, the date of her first offspring is recorded and included in the analysis as a variable. It was found upon analysis that the age and the form of the mother were not significant variables in this experiment.

The F values obtained using a Least-Squares Analysis of Variance for the various treatments are presented in Table V. When apterous versus alate progeny were compared for all the generations reared in

TABLE III. THE EFFECT OF TREATMENTS OVER 11 GENERATIONS ON THE PRODUCTION OF APTEROUS OFFSPRING - 16 HR. PHOTOPERIOD

Cage#	Treatment[a]	F_7 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother[b]	F_8 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_9 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{10} Generation # Alate	# Apterous	
27	ABA 6	0	10	257	B	0	10	263	B	0	8	269	B	0	27	
33	ABA 6	0	13	258	B	0	8	263	B	0	13	269	B	0	23	
28	ABA 60	0	10	259	B	0	8	263	B	1	10	270	B	14	14	
34	ABA 60	0	9	259	B	0	7	265	B	0	8	272	B	30	2	
29	GA_3 6	0	7	257	B	0	9	263	B	0	10	270	B	37	5	
35	GA_3 6	0	11	258	B	0	10	266	B	0	8	277	B	1	14	
30	GA_3 60	0	8	256	B	0	11	262	B	0	9	267	B	0	26	
36	GA_3 60	0	9	259	B	0	7	265	B	0	9	274	B	0	24	
31	D H_2O	0	8	257	B	0	12	264	B	0	8	270	B	1	20	
37	D H_2O	0	7	259	B	0	7	266	B	2	8	273	B	0	7	
32	T H_2O	0	9	257	B	0	10	263	B	0	6	270	B	0	31	*
38	T H_2O	0	5	258	B	0	8	264	B	0	7	*	*	*	*	

Cage#	Treatment	F_{11} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{12} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{13} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother
27	ABA 6	12	11	289	B	62	8	297	B	64	20	306	A
33	ABA 6	8	12	288	B	41	0	295	B	0	43	301	A
28	ABA 60	0	19	289	A	11	35	297	B	0	44	306	A
34	ABA 60	0	14	296	A	0	49	301	B	28	13	308	B
29	GA_3 6	0	31	288	A	15	16	295	B	52	11	305	B
35	GA_3 6	0	35	291	B	0	42	298	B	3	23	305	B
30	GA_3 60	8	15	291	B	0	28	299	B	2	33	304	B
36	GA_3 60	0	23	289	B	24	22	297	B	7	23	304	A
31	D H_2O	0	17	291	B	16	21	297	B	22	15	307	C
37	D H_2O	22	3	289	B	2	30	297	A	35	5	307	B

*-Data Unavailable

Cage#	Treatment	F_{14} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{15} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{16} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{17} Generation # Alate	# Apterous	Form of Mother
27	ABA 6	3	23	314	A	14	18	318	B	51	7	323	B	33	4	A
33	ABA 6	38	6	310	B	21	12	315	A	5	55	319	C	37	25	B
28	ABA 60	4	29	312	B	34	28	317	B	24	14	320	B	31	4	C
34	ABA 60	11	19	314	B	3	38	318	C	11	33	320	B	38	9	B
29	GA_3 6	10	14	311	C	37	20	318	A	20	18	321	A	22	27	B
35	GA_3 6	7	28	310	A	7	29	315	B	3	39	317	B	16	41	B
30	GA_3 60	24	14	308	B	7	47	318	B	19	29	324	B	41	14	B
36	GA_3 60	23	19	308	C	7	28	318	B	7	21	322	B	9	42	B
31	D H_2O	19	20	316	C	17	13	319	B	50	39	324	A	52	26	B
37	D H_2O	12	42	316	A	5	39	320	B	27	22	325	B	32	11	B

TABLE IV. THE EFFECT OF TREATMENTS OVER 8 GENERATIONS ON THE PRODUCTION OF APTEROUS OFFSPRING - AMBIENT PHOTOPERIOD.

Cage#	Treatment	F_7 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_8 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_9 Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{10} Generation # Alate	# Apterous
25a	ABA 6	0	3	261	B	1	10	276	B	0	1*	278	A	0	13
31a	ABA 6	0	8	260	B	0	5	269	B	0	8	278	B	3	12
26a	ABA 60	0	5	262	B	0	8	271	B	0	3	281	B	0	7
32a	ABA 60	0	5	259	B	0	6	267	B	0	9	275	B	1	20
27a	GA_3 6	0	3	259	B	0	10	268	B	1	10	281	B	1	3
33a	GA_3 6	0	9	261	B	0	9	273	B	1	2	319*	B	0	1
28a	GA_3 60	0	4	261	B	0	9	269	B	0	10	278	B	0	13
34a	GA_3 60	0	8	261	B	0	4	273	B	0	10	284	B	6	2
29a	D H_2O	0	7	261	B	0	13	269	B	3	13	280	B	2	2
35a	D H_2O	0	14	260	B	0	8	271	B	1	9	288	B	0	10
30a	T H_2O	1	8	263	B	0	6	272	A	0	8	284	B	20	4
36a	T H_2O	0	10	262	B	1	7	276	B	0	6	285	A	1	1

*-Adult wandered off of the plant and was replaced by an adult of the same generation and treatment.

Cage#	Treatment	Date of 1st Offspring	Form of Mother	F_{11} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{12} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother	F_{13} Generation # Alate	# Apterous	Date of 1st Offspring	Form of Mother
25a	ABA 6	292	B	0	12	301	B	27	7	313	B	70	3	323	A
31a	ABA 6	290	B	12	23	299	B	39	1	309	C	28	3	316	A
26a	ABA 60	289	B	0	7	299	B	3	5	309	B	13	2	*	B
32a	ABA 60	291	B	14	1	300	B	4	0	*	A	*	*	*	*
27a	GA_3 6	290	A	6	6	305	C	18	12	316	A	14	13	325	C
33a	GA_3 6	290	A	12	0	305	B	5	11	315	A	29	7	325	C
28a	GA_3 60	290	B	13	1	305	B	10	3	*	B	*	*	*	*
34a	GA_3 60	293	B	7	11	305	B	41	22	316	C	6	0	334	A
29a	D H_2O	288	B	13	13	298	C	42	4	311	C	7	0	*	C
35a	D H_2O	296	A	33	0	*	B	*	*	*	*	*	*	*	*
30a	T H_2O	297	B	13	9	305	A	32	5	319	C	12	2	332	A
36a	T H_2O	*	B	*	*	*	*	*	*	*	*	*	*	*	*

Cage#	Treatment	F_{14} Generation # Alate	# Apterous	Form of Mother
25a	ABA 6	14	31	C
31a	ABA 6	30	1	A
26a	ABA 60	*	*	*
32a	ABA 60	*	*	*
27a	GA_3 6	40	14	C
33a	GA_3 6	20	8	C
28a	GA_3 60	*	*	*
34a	GA_3 60	*	*	*
29a	D H_2O	*	*	*
35a	D H_2O	*	*	*
30a	T H_2O	33	0	A

*-Data Unavailable

a - ABA = abscisic acid and GA stands for gibberelic acid.

b - A = alate; B = apterous; C = alate + apterous.

the 16-hour photoperiod, significantly more apterous offspring ($P = 0.01$) were obtained. This is consistent with results obtained from rearing aphids in long photoperiods (16-hour) in uncrowded conditions. Significant treatment effects were obtained in the F_{10} generation ($P = 0.01$) indicating that plant hormones affect wing dimorphism in the aphid R. padi.

Figures 1-5 are graphical portrayals in an attempt to further elucidate these results. The results of all treatments given in the ambient photoperiod, illustrated in Figure 1 , indicate that insufficient data were obtained to illustrate any distinct trends. The mean percent apterous offspring / generation in each treatment administered in the 16-hour photoperiod is shown in Figure 2. Figures 3-5 are treatments of the 16-hour photoperiod, separated out to indicate trends.

Young foliage is known to direct wing dimorphism toward an apterous course, while maturing or senescent tissue allows for the production of alate offspring. It has also been shown that in young, growing tissue, levels of GA are high, whereas in maturing or senescent tissue, levels of ABA are high (Leopold and Kriedmann, 1975). If these two plant hormones have an influence on wing dimorphism, it would be expected that high levels of GA would initiate the production of apterous offspring, while high levels of ABA would allow for the production of alate offspring.

TABLE V. F VALUES OBTAINED FROM THE DATA USING LEAST-SQUARES ANALYSIS.

Generation	16 hr. Photoperiod F Value	Ambient Photoperiod F Value
F_{10}	20.727**	.625
F_{11}	.740	.509
F_{12}	3.286	.387
F_{13}	.465	.000
F_{14}	.661	D/U
F_{15}	.181	D/U
F_{16}	1.866	D/U
F_{17}	1.832	D/U

Comparison of ambient and 16 hr. photoperiod combined = .628

Comparison of apterous v.s. alate production in 16 hr. photoperiod = 9.24**

The significance obtained at the F_{10} generation in the 16-hour photoperiod regimen tends to support this hypothesis. Aphids had continued along an apterous course of development, as would be expected due to the influence of photoperiod, until the F_{10} generation was reached. At this time, the development of alate offspring became apparent in the higher concentration of ABA and the lower concentration of GA_3, while the other treatments continued along an apterous course of development. It is at the F_{10} generation that the first treatment effects would be observed due to the 3 generations necessary to remove the effects of previous conditions. The highly variable data obtained in the ambient photoperiod could be attributed to the occurrence of powdery mildew, or to changes in the physiology of the host plant that accompany decreasing photoperiods.

No significant treatment effects were obtained, perhaps in part, because of the high variance in that regimen. That no significant treatment effects were obtained in the generations after the F_{10} raises many questions that may be explained in part by the Figures 2 to 5.

Two observations become apparent in Figure 2. First, that there were similarities between certain treatments and second, that a peak of alate production occurred in all the treatments at different generations.

Figure 1. Percent apterous offspring that occurred in each treatment in the ambient photoperiod regimen over 8 generations.

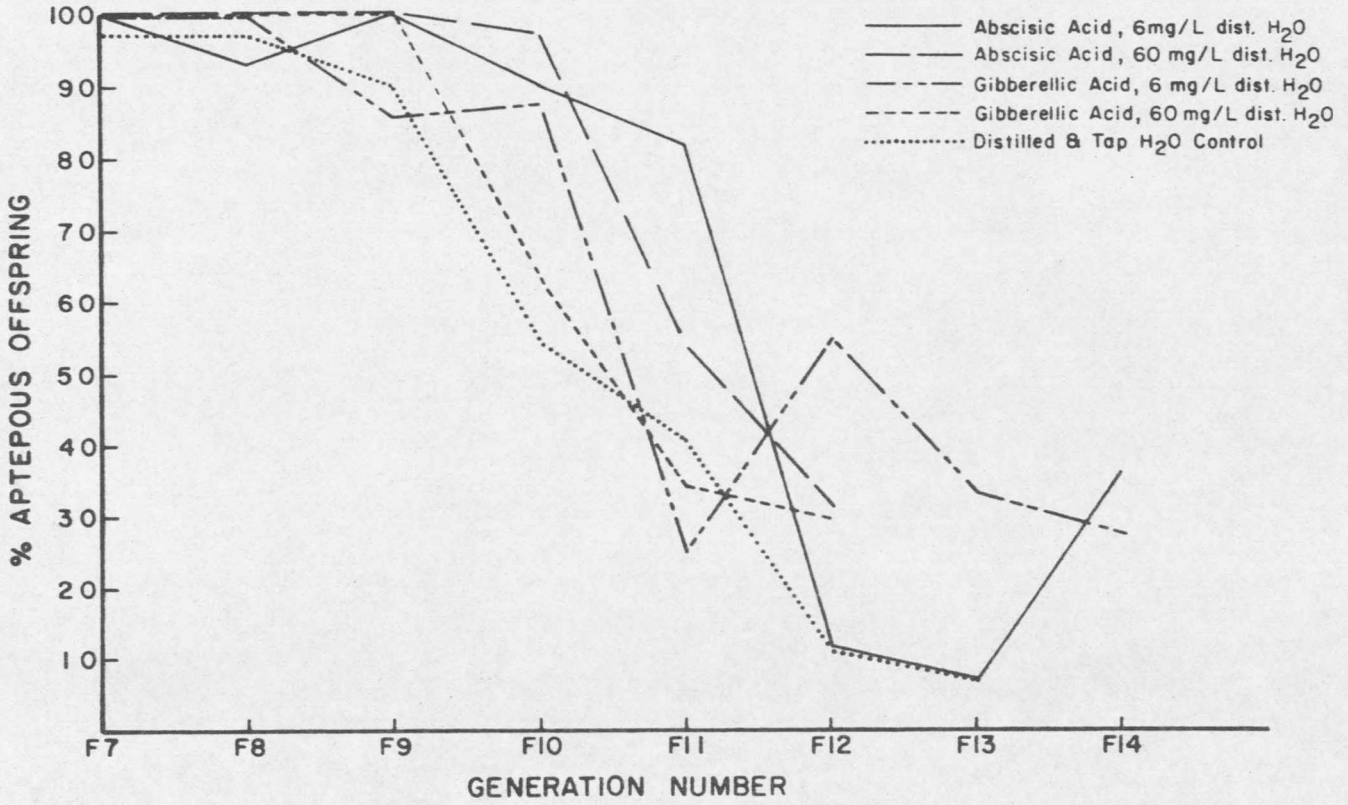
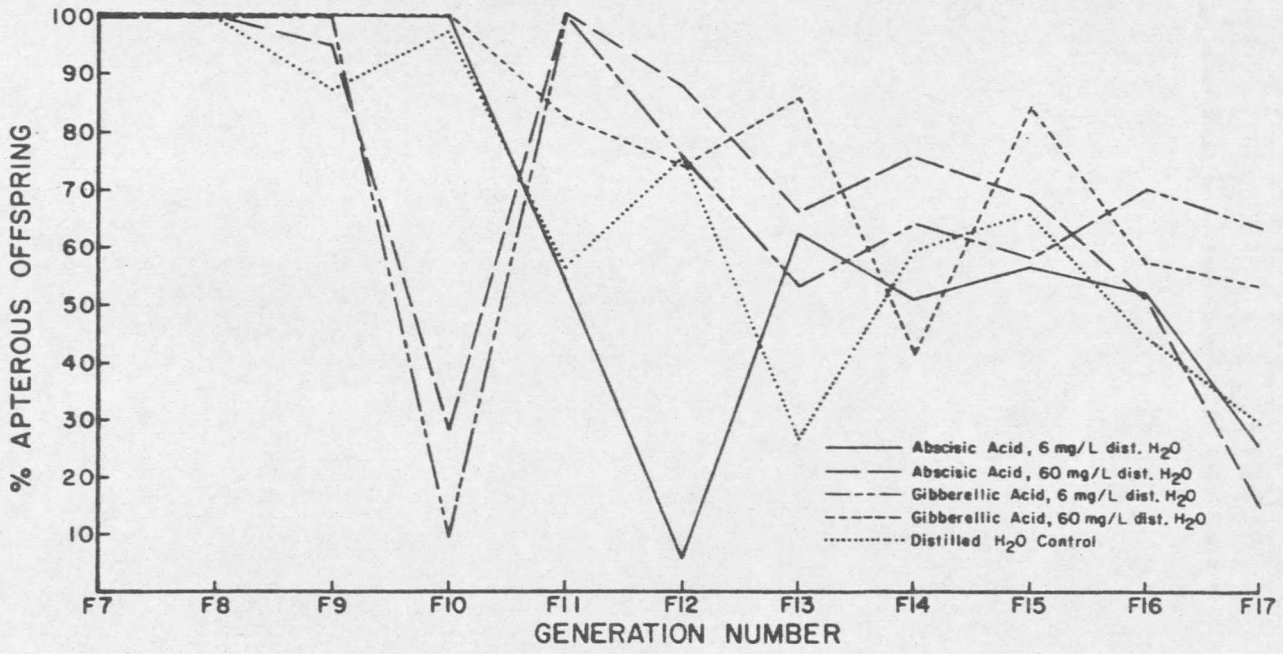


Figure 2. Percent apterous offspring that occurred in each treatment in the 16 hour photoperiod regimen over 11 generations.



The similarity between the two treatments - ABA-60 and GA_3-6 are illustrated in Figure 3.. Aphids under these two treatments followed the same trend with respect to wing dimorphism until the F_{15} generation was reached, after which the GA_3-6 treated aphids followed an apterous course of development while the ABA-60 treated aphids began producing predominatly alate offspring. As it is sometimes the case that when ABA is found at high levels in plant tissue GA occurs at low levels and vice versa (Thomas et. al. in 1965; Chrispeels and Varner in 1966; cited in Leopold and Kriedmann, 1975), therefore, it is not surprising that a similar behavioral trend was experienced by the aphids under the treatments, ABA-60 and GA_3-6.

A similar trend between the GA_3- 60 treatment and the control is shown in Figure 4. When compared to the control, the GA_3-6 treatment seems to have produced a slightly apterizing effect with the fluctuations delayed by a generation. As higher levels of GA_3 would be expected to influence wing dimorphism towards an apterous course and as the control was under conditions conducive to apterous production (16-hour photoperiod), it seems reasonable that these two regimens should be similar, although the response of one is delayed one generation.

The effects of the two concentrations of ABA on wing dimorphism as shown in Figure 5 may be interpreted to mean that two generations more were needed in the ABA 6 treatment before the levels of ABA built up enough to cause the production of alates.

Figure 3. Percent apterous offspring that occurred in the treatments ABA-60 and GA_3-6 in the 16 hour photoperiod regimen over 11 generations.

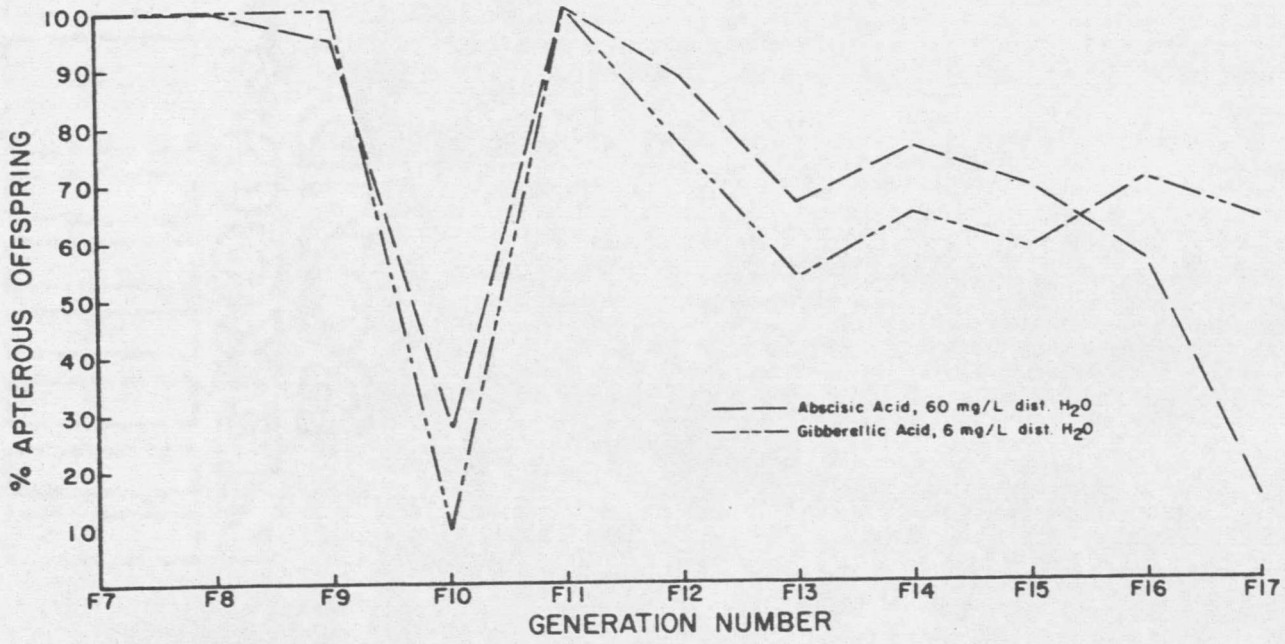


Figure 4. Percent apterous offspring that occurred in the GA_3-60 treatment and the control in the 16 hour photoperiod over 11 generations.

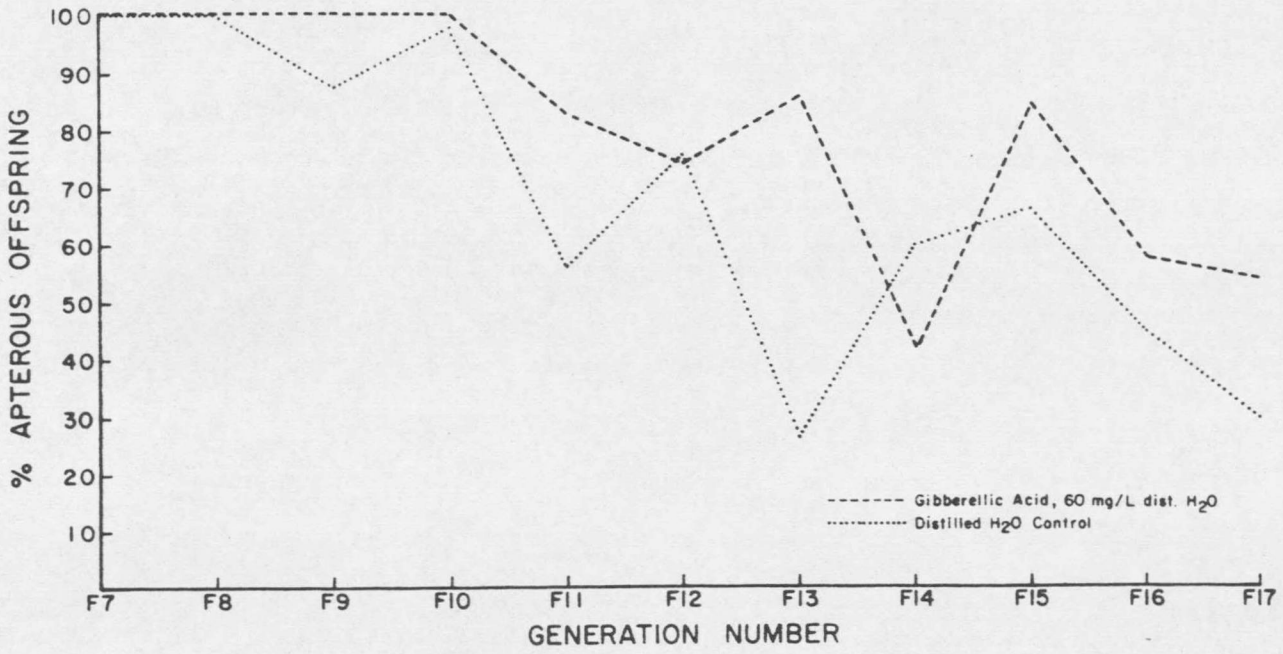
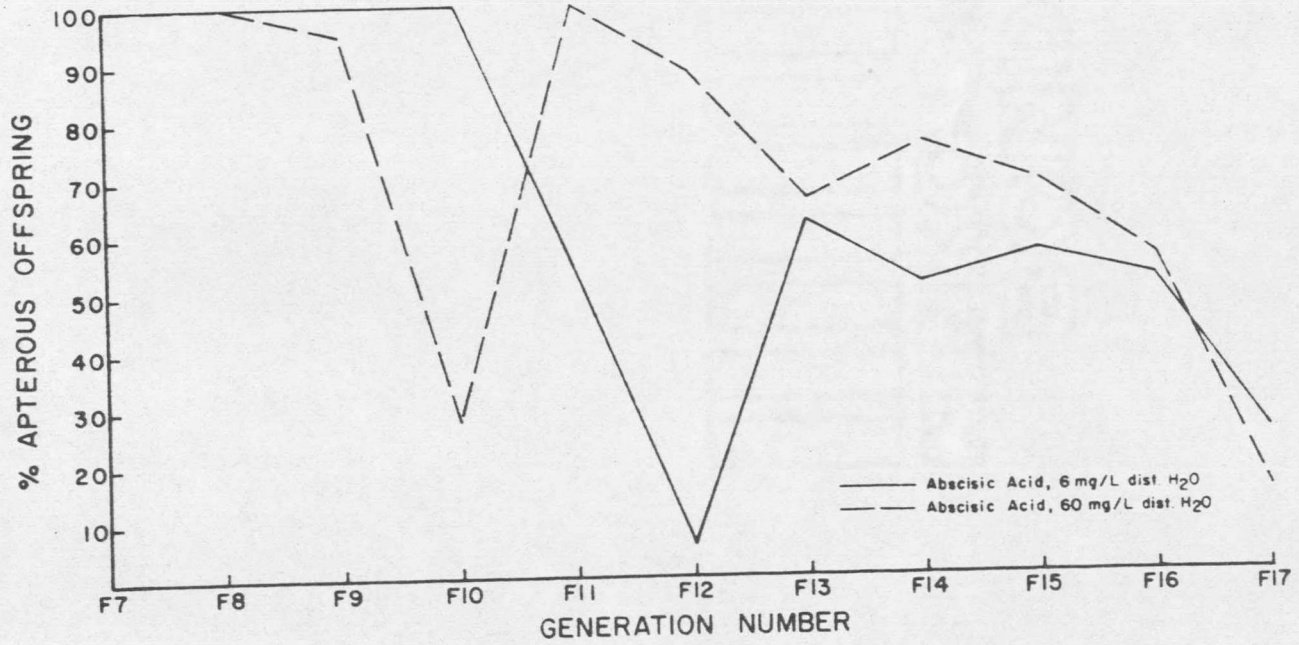


Figure 5. Percent apterous offspring that occurred in the treatments ABA-6 and ABA-60 in the 16 hour photoperiod regimen over 11 generations.



That distinct morphological changes occurred in response to the treatments administered in the 16-hour photoperiod is evident by the figures presented. Whether the hormone treatments are somehow compensated for by the aphid after prolonged exposure, or if the variability inherent in the experiment is responsible for the lack of significance obtained after the F_{10} generation, is not known.

Corpus Allatum

The calculated volumetric measurements of the corpus allatum glands of thirteen aphids that produced mostly apterous offspring are presented in Table VI. These measurements in thirteen adults that produced mainly alate progeny are illustrated in Table VII. When the volumes of these two groups were compared by analysis of variance, they were found to differ significantly ($F = 4.98318$; $P = 0.035$).

The high correlation between the corpus allatum size of the mother and form production in the offspring suggests there is prenatal, rather than postnatal, determination of wing form in R. padi. Since the size of the corpus allatum gland is thought to indicate its activity (White, 1968), it seems likely that the production of apterous progeny is under the control of juvenile hormone. It must be kept in mind, however, that there is controversy regarding the validity of using the volume of the corpus allatum as an indicator of its activity (Staal, 1961; Gillot and Dogra, 1972).

TABLE VI. MEASUREMENT OF THE CORPUS ALLATUM GLAND IN ADULT APHIDS THAT PRODUCED PREDOMINANTLY APTEROUS OFFSPRING.

Date 1977 Fixed	Pot#	Aphid Description	# & Form of Progeny # alate	# apterous	Volume$_3$ of CA(μ^3)
310	27	F_{13} alate	3	23	45.6
314	20	F_{14} apterous	7	47	46.1
282	35	F_{9} alate	1	14	28.8
307	28	F_{13} apterous	4	29	72.3
310	35	F_{13} apterous	7	29	66.8
304	35	F_{14} alate	7	28	62.8
292	35	F_{11} apterous	0	42	61.6
314	27	F_{14} apterous	14	48	47.6
304	28	F_{12} alate	0	44	68.7
307	37	F_{13} apterous	12	42	43.2
223	12	F_{2} apterous	0	19	83.8
231	11a	F_{1} apterous	2	8	66.8
230	12	F_{3} apterous	0	24	56.6

TABLE VII. MEASUREMENT OF THE CORPUS ALLATUM GLAND IN ADULT APHIDS THAT PRODUCED PREDOMINANTLY ALATE OFFSPRING.

Date 1977 Fixed	Pot#	Aphid Description	# & Form of Progeny # alate	# apterous	Volume$_3$ of CA(μ^3)
314	34a	F_{12} alate	6	0	26.7
310	29	F_{14} alate	37	20	47.7
305	27a	F_{11} alate	18	12	47.1
304	32a	F_{11} alate	4	0	26.2
311	26a	F_{12} apterous	13	2	56.5
311	31a	F_{12} alate	28	3	31.4
304	36	F_{13} apterous	23	19	47.1
306	34a	F_{13} apterous	41	22	69.1
304	30	F_{13} apterous	24	14	44.5
310	33	F_{14} alate	21	12	52.7
222	10a	F_{2} alate	23	7	43.2
231	16a	F_{2} apterous	10	0	56.5
229	10a	F_{13} alate	30	0	47.6

Analysis of Variance Comparison of the Above Data:
F = 4.98318 = P = 0.3519

DISCUSSION

Aphids are said to begin development as presumptive alatae, so that apterae and intermediate forms are those which are thought to have been diverted from the alate course during development. Phenological signals such as photoperiod, temperature, humidity, crowding and nutrition trigger development of the embryo towards an alate or apterous course. This developmental determination as a response must occur indirectly through the reproductive physiology of the mother, or by a direct response through the physiology of the offspring. Lees (1964) with the use of pinpoint light sources, showed that photo-receptors are located on the head of the adult aphid, Megoura viciae Buck. and that form determination was brought about through the reproductive physiology of the mother. Other researchers (Noda, 1958; Kawada, 1965; White, 1971) suggest that offspring have the ability to determine their own wing form up to 48 hours after birth.

The correlation obtained in this paper between the corpus allatum size of the mother and form production in the offspring suggests that there may be prenatal determination of wing form in Rhopalosiphum padi (L.). Since size of the corpus allatum gland has previously been used as a parameter of its activity (White, 1968), this would suggest that the production of apterous progeny may be under the control of juvenile hormone.

The results of the photoperiod study indicate that R. padi reacts in a manner similar to other species of heteroecious aphids with regard

to the influence of photoperiod on wing dimorphism. Increasing photoperiods initiate the production of apterous progeny, while decreasing photoperiods cause the production of alate offspring. If, as was suggested by the corpus allatum study, the production of apterous progeny is under the control of juvenile hormone, then longer photoperiods must, in some manner, activate the corpus allatum. It is still a matter of debate, however, whether the photoperiod acts directly on the aphid or indirectly through nutritional changes in the aphids' host plant to produce this response.

Variable photoperiods, as well as other environmental stimuli, may bring about changes in the levels of hormones in the host plant. Treatment with the plant hormones GA_3 6 mg/L distilled H_2O and ABA 60 mg/L distilled H_2O elicited a significant effect at the F_{10} generation in the 16-hour photoperiod, suggesting that these hormones affect wing dimorphism in R. padi. The treatments ABA 60 and GA_3 6 appear to influence wing dimorphism towards an alate course of development, while GA_3 60 seems to cause an apterizing effect. Thus, the treatment with ABA 60 and GA_3 6 caused a reversal in wing form production that was obtained under the influence of photoperiod alone. This suggests that these plant hormones interferred with the normal response to photoperiod. This interference could be achieved in several ways; 1) low concentrations of GA and high concentrations of ABA may mimic the plant conditions under shorter photoperiods, thus eliciting the production of

alate progeny, 2) these plant hormones may inhibit the photoperiod response, or 3) the stimulus provided to the aphid by these plant hormones may be stronger than that provided by photoperiod. Since GA and ABA occur naturally in the host plant, and their titers may be linked to photoperiod, it seems unlikely that these plant hormones would significantly alter the photoperiod response. In response to short photoperiods, however, GA may occur at low concentrations, while ABA may be at higher concentrations in the plant, so that it seems likely that this condition mimics that of a short photoperiod. In any case, it seems apparent that because these plant hormones can reverse the response of photoperiod, that the photoperiod response must be manifested in the aphids through changes in its host plant.

Mittler (1972) suggested that a yet unknown apterous-promoting principle existed which was of a chemical nature and probably ingested. It could be postulated from the present research that GA in relatively high concentrations either produces in the plant a substance, or is itself the "apterous-promoting principle". This "apterous-promoting principle" must act in turn to activate the corpus allatum gland to secrete juvenile hormone. Relatively high concentrations of ABA on the other hand, elicit a response in the aphid that is opposite to that obtained with high concentrations of GA. It may be postulated that ABA itself inhibits or produces in the plant a substance which inhibits

secretion by the corpus allatum gland or the activity of the juvenile hormone.

Further investigation into the role of plant hormones on the determination of wing dimorphism in aphids seems warranted.

SUMMARY

Increasing photoperiods in Rhopalosiphum padi (Linn.) were found to direct wing dimorphism towards an apterous course, while decreasing photoperiods directed wing formation of offspring towards an alate course of development. As plant hormones can reverse the response of photoperiod, the photoperiod response must be manifested in the aphids through changes in its host plant.

The study of the effects of plant hormones on wing dimorphism elicited a significant treatment effect in the F_{10} generation in the 16-hour photoperiod regimen. ABA-60 and GA_3-6 appear to cause the production of alate progeny, while GA_3-60 seems to initiate the production of apterous offspring.

The correlation obtained in this paper between the corpus allatum size of the mother and form production in the offspring suggests that there may be prenatal determination of wing form in R. padi. Since size of the corpus allatum has been suggested to indicate its activity, the apterous production of progeny may be favored by high titres of juvenile hormone in the mother.

APPENDIX

MODIFICATIONS OF TECHNIQUES FOR THE ADAPTATION TO APHID TISSUES

Double Embedding with Methyl Benzoate Celliodin (Humason, 1962)

80% ETOH	5 minutes
95% ETOH	2 changes 5 minutes each
100% ETOH	2 changes 5 minutes each
Toluene	2 changes 15 minutes each
Methyl Benzoate Celloidin	24 hours
Toluene	2 changes, 1 hour each
½ paraffin and ½ Toluene	2 changes, 1 hour each

Embed in paraffin.

Ewen's Aldehyde-Fuchsin Staining Technique

Toluene	5 minutes
Toluene	5 minutes
½ Toluene and ½ 100% ETOH	5 minutes
100%	1 minute
100%	1 minute
95%	1 minute
80%	1 minute
70%	1 minute
50%	1 minute
Dist. H_2O	1 minute
Oxidize in acid permaganate	2½ minutes

Rinse in dist. H_2O	
Decolorize in 2½% NaHS	40 seconds
Rinse in dist. H_2O	
30% Rinse	
70% Rinse	
Aldehyde-Fuchsin	15 minutes
95%	Wash
Differentiate in acid alcohol	
70%	Rinse
30%	Rinse
Dist. H_2O	Rinse
Mordant	90 minutes
Dist. H_2O	Rinse
Counterstain	20 hours
0.2% acetic acid	Rinse
Hydrate	
Take through Toluene	
Mount in Adam's Histoclad	

Sections must be stained as soon as possible after they are cut.

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