JUVENILE HORMONE CONTROL OF DEVELOPMENT OF

SELECTED TISSUES IN THE MIGRATORY GRASSHOPPER,

Melanoplus sanguinipes (Fabr.) (ORTHOPTERA:ACRIDIDAE)

by

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ABSTRACT

Changes in total body weight and in dry weights of the internal organs indicated that male and female adults of *Melanoplus sanguinipes* undergo a biphasic growth pattern. Regression analyses indicated that the overall growth rates were comparable in the two sexes during the somatic growth phase but differed markedly during the reproductive phase. Reasons for these differences are discussed in light of the behavior and physiology of the sexes.

Head width, tibia length, tegmina length, and dry weights of gonads, fat body, and flight muscles were highly correlated in normal adults. This indicated that growth patterns were highly coordinated within individual insects. Fluctuations in the dry weights of the fat body and flight muscles during reproductive development indicated that these tissues were a source of protein for the developing ovaries or accessory glands. The similarity in the pattern of changes in males and females indicated that development may be synchronized between the two sexes. Factors contributing to this apparent synchrony are discussed.

Depending upon the time of application, both the anti-allatotropin, precocene II and the juvenile hormone analog (JHA), R-20458 have been shown to drastically alter the development of various tissues in *M. sanguinipes*. The present studies substantiate previous reports that JH regulates the development of the fat body and gonads. In addition, JH has been shown to regulate metamorphosis,

somatic growth, coloration, wing length, and development of the
flight muscles and fat body during the fifth instar. Precocene applied
to fourth instars caused precocious metamorphosis and the production
of diminutive adults. However, nearly normal development was produced
in precocene-treated insects when R-20458 was applied 4 days later.
Later JHA treatments resulted in the production of nymphal-adult
intermediates. Intermediates were also produced when JHA alone was
applied to fifth instars. However, specific effects depended upon
precise application time. Supernumerary molting occurred only in
insects treated with JH during the middle of the fifth instar stadium.
Therefore, absence of JH at this precise time seems to be necessary
to permit the imaginal molt. Green-colored and short-winged adults
characteristic of locust *solitarious* phase, were produced when JHA
was applied at certain times within the fifth instar. JHA application
to fifth instars resulted in a significant reduction in wing length
and in dry weight of the flight muscles. Flight muscles were
sensitive to JHA throughout the fifth stadium whereas wing length
was only significantly affected by JHA during certain periods of
the stadium. Therefore, the flight muscles must be developing
separately from the wings. JHA applications to precocious adultoids
were too late to change the commitment of flight muscles in either
sex, or of the male gonads. However, the fat body of males and
females, and the female gonads were still susceptible to JHA at this
time, and the precocene-induced sterility of females was reversed.

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INTRODUCTION

A. *Pest Status*

The migratory grasshopper, *Melanoplus sanguinipes* (Fabr.),
is generally regarded as the most widespread and destructive
grasshopper species in North America (Pickford and Mukerji, 1974;
Hewitt, 1977; Uvarov, 1977). *M. sanguinipes* damages cereal grains,
vegetables, forages, and even the leaves and bark of fruit trees
(Metcalf and Flint, 1962; Parker and Connin, 1964). Locusts, close
relatives of *M. sanguinipes*, are a major world pest that have caused
periodic crop decimation and famine for centuries (Baron, 1972; Hill,
1975).

Improved methods of grasshopper and locust control are
currently needed (Uvarov, 1977). The International Study Conference
on the Current and Future Problems of Acridology recommended the
study of insect hormones as possible potent, environmentally-compatible
insecticides and particularly emphasized the potential of locust
control through phase manipulation with juvenile hormone (Anonymous,
1970).

B. *M. sanguinipes - Grasshopper or Locust?*

The migratory grasshopper may actually be a locust.
Grasshoppers are distinguished from the locusts in the same family by
their inability to transform into a gregarious, highly mobile phase
during their life cycle. While locusts migrate in huge swarms over

vast distances and eat most of the plants in their path, grasshoppers remain relatively localized and solitary. The name "migratory grasshopper" shows the ambivalence of scientists regarding the status of *M. sanguinipes*. The species does not show the two extreme morphological forms common in locusts, but is capable of migrations of 10 to 50 miles per day (Willis, 1939) for distances of up to 575 miles (Riegert, 1962). Mass migrations of *M. sanguinipes* have also been reported by Bethune (1874), Gurney (1952), and Parker *et al.* (1955). In addition, the genus *Melanoplus* is categorized by Rowell (1967) as containing locust species. Members of the genus exhibit the locust characteristic of green/brown polymorphism, at least in the haemolymph of individuals with high corpora allata activity (Pfeiffer, 1945).

A closely related species, the Rocky mountain locust, *Melanoplus spretus* (Walsh), was the predominant species during the early 1900's but appears to have become extinct. However, some authors (Buckell, 1972; Faure, 1933; Huffaker and Messenger, 1976) believe it may be a rarely-occurring phase of *M. sanguinipes*. *M. spretus* is very similar to *M. sanguinipes* but has longer, broader wings and a darker coloring (Helfer, 1953). Perhaps *M. sanguinipes* is a locust whose extreme gregarious phase is not triggered by present North American conditions.

C. *Phase Polymorphism in Locusts*

Locust phase theory, first expounded by Uvarov (1921, cited by Ordish, 1976) explains the natural polymorphism of many acridid populations and forms the foundation of modern locust control. Uvarov observed two extreme physiological states or phases, a solitary and a gregarious phase. Although not common to all locust species, the two phases exhibit a variety of characteristics (Table I). Locusts in the *solitaria* phase display grasshopper-like behavior and morphology including a more arched pronotum, shorter wings, and larger femur/head capsule ratio than *gregaria* adults. *Solitaria* are often light-colored or even green, feed and develop as isolated individuals and make only short flights. Rates of feeding, and development in *solitaria* are slower than in *gregaria*, and *solitaria* locusts sometimes have an extra nymphal instar. Egg-laying is delayed in *solitaria*, but the females are larger and their fecundity is higher than in *gregaria* locusts (Kennedy, 1961).

Phase *gregaria* exhibits long-distance migrations. These flights begin under suitable weather conditions when the morphology and behavior of the new locust generation have begun to change from the solitary to the gregarious form. Dark-colored gregarious nymphs begin marching and are joined by similar individuals until a large hopper band has formed. After the nymphs molt into long-winged adults, they mill around for several days, taking short practice flights until they are all ready for flight (Chapman, 1969). Swarms form and the

TABLE I: Phase characteristics of locusts

Phase character	Effect	Genus or species	References

behavior	solitaria
	  limited flight; no grouping or
	    marching
	  individuals avoid each other
	gregaria
	  mass flight; march in groups
	  individuals stay together
		general	Kennedy (1961)
			Gillett (1978)

color	solitaria
	  green, yellow, or light beige
	gregaria
	  tan to dark brown or black
	  may have pinkish background color
	    or yellowing
		Locusta
		Schistocerca
		Locustana
		grasshopper spp.
			Cassier (1966)
			Kennedy (1961)
			Gillett (1978)
			Rowell (1967)

morphology 1. femur/head capsule	larger in *solitaria*; smaller in *gregaria*	*Schistocerca* *Locusta*	Gillett (1978) Nolte (1976)

2. wing length; length
   of elytron/hind
   femur ratio
	solitaria *gregaria*
	shorter wings longer wings
	slightly lower slightly higher
	  E/F ratio E/F ratio
		Zonocerus
		  variegatus
		Schistocerca
		Locusta
			McCaffery & Page (1978)
			Kennedy (1961)
			Fuzeau-Braesch &
			  Nicholas (1970)

3. pronotum	arched in *solitaria*		

compound eyes and
  time of flight
	solitaria *gregaria*
	eyes have light- eyes are dark
	  colored spots and a solid
	  or bands color
	fly at night day flight
		Schistocerca
		Locustana
		Locusta
			Nolte (1978)
			Cassier (1965)
			Davey (1959)

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TABLE I: *(continued)*...

Phase Character	Effect	Genus or species	References
development			
1. egg hatching	*solitaria* slower than *gregaria*	*Schistocerca* *Locustana*	cf. Kennedy (1961)
2. maturation rate	*solitaria* slower than *gregaria* *solitaria* sometimes has an extra instar	*Schistocerca* *Locusta* *Nomadacris*	
3. young hoppers	*gregaria* nymphs are heavier than *solitaria*, and contain more dry matter	*Schistocerca* *Nomadacris* *Locusta*	
4. early adulthood	*gregaria* feed more rapidly than *solitaria* and have a higher metabolic rate	*Schistocerca* *Nomadacris*	
5. sexual dimorphism	*solitaria* have bigger ♀ and smaller ♂ than *gregaria* Therefore in *gregaria* there is a smaller difference between the sexes.	*Schistocerca* *Nomadacris*	
6. maturity	a) *gregaria* begin oviposition later than *solitaria* b) *gregaria* begin oviposition earlier than *solitaria*	*Locusta* *Schistocerca* *Nomadacris*	
7. fecundity	lower in *gregaria* than in *solitaria*	*Locusta* *Nomadacris* *Schistocerca*	

locusts fly together for hundreds of miles. During the migration, the female grasshoppers or locusts contain undeveloped oocytes, whereas the males are usually older and more sexually mature (Davey, 1959; Johnson, 1969; Chapman *et al.*, 1978). Migrations usually continue until the females begin to form eggs and leave the swarm in search of oviposition sites, when the band disperses.

D. *Environmental Factors Influencing Locust Phase Determination*

Locusts benefit substantially by their ability to change state in response to their fluctuating environment. In wet weather, when food is plentiful, they are dispersed amongst the vegetation producing large numbers of eggs and growing slowly into large, pale-colored or green adults which are well camoflaged in their surroundings. When the climate becomes hot and dry, the locusts numerous, and food plants scarce, locusts migrate to more favorable areas. The long wings, rapid development, and organized behavior of *gregaria* are ideally suited to large-scale dispersal. Within a few generations locusts adapt to their new conditions.

Locust phases are determined by both genetics and environment. Although selective breeding can bring about distinct *solitaria* and *gregaria* lines within four generations, the same variation in phase character can be induced within one generation by environmental stimuli such as rearing density. The altered state is then maternally inherited (c.f. Kennedy, 1961).

Enviromental conditions triggering phase change are listed in Table II. Not surprisingly, form *gregaria* develops when the insects are densely populated. High temperatures, low relative humidity, and long days also favor phase *gregaria*, while *solitaria* is encouraged by the opposite conditions. Even the presence of faeces from certain other locust stages has been found to influence phase determination in caged nymphs (Gillett and Phillips, 1977). In addition, locusts are phase-sensitive to carbon dioxide levels (Fuzeau-Braesh and Nicolas, 1970; Doane, 1973) and to diet. Brett (1947) produced brachypterous *M. sanguinipes* by rearing the grasshoppers on lucerne at low temperatures and high humidity (Uvarov, 1966). Perhaps *M. spretus* was just an extreme phase of *M. sanguinipes* that was no longer favored by the new food plant communities resulting from the human settlement of North America.

Changes in diet can alter the hormonal balance within insects and may increase or decrease the likelihood of migration. According to Staal (1967), *"the discovery that particular plant substances simulate the effects of juvenile hormone and molting hormone of the insects may throw new light on the migratory behavior of many phytophagous insects"*.

E. *Juvenile Hormone Control of Selected Aspects of Insect Development*

Environmental factors appear to trigger hormonal responses that initiate phase change. The *solitaria* phase is thought to be a

TABLE II: Environmental factors influencing locust phase determination

Phase character	Effect	Genus or species	References
crowding	extreme solitarius phase at very low density extreme gregarious phase at very high density	*Zonocerus*	McCaffery & Page (1978) Chapman *et al.* (1978)
	intermediate forms at intermediate densities	*Schistocerca* *Locusta*	Kennedy (1961)
status of parents	*solitaria* parents produce a higher proportion of *solitaria* offspring than do the intermediates *gregaria* parents produce a higher proportion of *gregaria* offspring	*Nomadacris* *Locusta*	Kennedy (1961) Hunter-Jones (1958) Nolte (1976)
humidity	low humidity favors *gregaria* high humidity favors *solitaria*	*Zonocerus variegatus* *Locusta*	Chapman *et al.* (1978) Davey (1959) Doane (1973)
temperature	low temperatures favor *solitaria* high temperatures favor *gregaria*	*Schistocerca*	Gillett (1978) Albrecht *et al.* (1978)
photoperiod	short days favor *solitaria* long days favor *gregaria*	*Schistocerca*	Gillett (1978) Albrecht *et al.* (1978) Doane (1973)
food plants	various plants favor one phase	*Schistocerca* *M. sanguinipes*	Staal (1961) McCaffery & Page (1978) Uvarov (1966) Brett (1947)

(continued)...

TABLE II: *(continued)*...

Phase character	Effect	Genus or species	References
carbon dioxide	high levels of carbon dioxide favor *solitaria*	*Locusta*	Doane (1973) Fuzeau-Braesch & Nicholas (1970)
faeces	faeces from crowded adults increase *solitaria* characters in nymphs faeces from crowded numphs increase *gregaria* characters in numphs	*Schistocerca*	Gillett & and Phillips (1977)

permanently neotenized form arising from high juvenile hormone (JH) titres during the last nymphal stage (Kennedy, 1961; Doane, 1973; Cassier and Delorme-Joulie, 1976). When locust nymphs receive corpora allata (CA) implants or are treated with juvenile hormone analogs (JHA), *solitaria*-like adults are produced (Joly, 1960; Staal, 1961; Doane, 1973). Conversely, solitary *Locusta* females with one CA removed produce progeny which show gregarious characteristics (Cassier, 1966; Doane, 1973). Phase coloration changes can also be induced by allatectomy (Kennedy, 1961), CA implants, and external application of JHA (Joly and Meyer, 1970; Nemec, 1970; Kruse Pedersen, 1978). Rowell (1967) reports that color change seems to be a general characteristic of the Acridoidea, and can be induced with high JH levels even in species where green individuals in the wild are rarely found (Pfeiffer, 1945).

(a) *Reproduction*

In many acridid species including *M. sanguinipes*, the CA have been shown to stimulate ovarian development (Gillott and Elliott, 1976; Elliot and Gillott, 1976, 1977, 1978, 1979). Since JH accelerates reproductive growth low JH levels in early adult migrants could explain the slow sexual maturation in the females of some locust species. Similarly, JH is also involved in the reproductive functions of male locusts. In *S. gregaria*, allatectomies performed on young males completely abolished male

sexual behavior (Loher, 1961; Pener, 1967). Evidence for CA control of the development and functioning of the male accessory glands has been demonstrated in *M. sanguinipes* (Gillott and Friedel, 1976a,b).

(b) *Wing Length*

"*It is well-known that JHA applied to insects at the preimaginal stage disturb the imaginisation of integuments and wing formation*" (Chudakova *et al.*, 1976). In the tropical pest grasshopper, *Zonocerus variegatus*, crowding or low JH levels during the last nymphal instar produced long-winged adults capable of long flights (McCaffery and Page, 1978). Allatectomy performed on 3-day-old fifth instars of *Z. variegatus* produced only long-winged adults (McCaffery and Page, 1978). In contrast, JHA applied to final instar *Locusta* and *Schistocerca* caused curly and/or shortened wings, the effects being dependent on precise timing of JHA application (Nemec, 1970).

(c) *Flight Muscle Development*

The role of the CA in insect flight muscle development remains uncertain (Gilbert and King, 1973). However, researchers have observed that "*maturation of flight muscles differs between species and sexes and may be under the control of the CA*" (Rockstein and Miguel, 1973). In the majority of species investigated, JH inhibits flight muscle development in nymphs

and encourages flight muscle degeneration in adults. In *H. cecropia*, formation of many adult tissues occurs during the first 2 days of pupal development, when JH levels are low. However, when CA are implanted during early pupation, flight muscle formation is suppressed (Williams, 1961). In *in vitro* experiments on various insects, JH suppressed imaginal wing disc development (Patel and Madhaven, 1969; Chihara and Fristrom, 1973; Benson and Oberlander, 1974; Oberlander and Silhacek, 1976).

Some insects normally exhibit wing-casting and/or flight muscle degeneration after the initial active adult flights give way to reproductive activity. However, in some of these insects flight loss can be prevented by removal of the CA. In the house cricket, *Acheta domestica*, allatectomies performed during the final nymphal instar prolonged flight muscle persistance in the adults (Chudakova and Bocharova-Messner, 1968b) and resulted in a permanent retention of flight ability (Chudakova and Gutmann, 1978). Conversely, JHA application caused rapid degeneration of the flight muscles in both nymphal and adult *Acheta* (Chudakova and Bocharova-Messner, 1968a, 1968b). Adult fire ants, *Solenopsis invicta*, failed to cast their wings or undergo normal flight muscle histolysis after allatectomy (Barker, 1979) but subsequent JHA application to the adults resulted in rapid flight muscle degeneration. Implantation of CA into adult female, *Dysdercus intermedius* (Edwards, 1970), also elicited flight muscle histolysis.

In various bark beetle species, JHA induce degeneration of the
adult flight muscles (Borden and Slater, 1968; Unnithan and
Nair, 1977).

F. *Mode of Action of JH on Flight Muscles*

(a) *Effect on Protein Content*

The diameter and protein content of the flight muscles
of some insects mirror their increasing flight capability during
early adulthood (Poels and Bennakkers, 1969; Bursell, 1973;
Panar and Nair, 1975; Baker, 1976). Protein, the major component
of muscle (Panar and Nair, 1975), is also affected by JH levels.
In Poels and Beenakkers' experiments (1969), protein accumulation
in adult locust flight muscles was greatly reduced, but not
totally blocked, by implanting active CA during the early fifth
instar. The likelihood of uneven release from the CA throughout
adult development makes it impossible to determine from their
experiment whether or not there is a critical stage during which
JH influences flight muscle development. Perhaps protein synthesis
in the flight muscles would not be affected if JH were applied at
the late fifth instar stage. However, since the CA caused the
greatest inhibition of flight muscle protein during the differentiation
stage (2 to 5-day-old adults), JH applications to newly-emerged
adults should be effective in reducing flight muscle protein content
unless the tissues are already committed at this time. The

sensitivity of the flight muscles of fifth instar nymphs to

CA implantation led Poels and Beenakkers (1969) to suggest that in

normal *Locusta*, flight muscle development only begins when the JH

titre is very low. The authors presume that JH titres in *Locusta*

are negligible throughout the fifth nymphal stadium. Capillary

gas chromatography with electron capture (Blight and Wenham,

1976a,b; Huibregetse-Minderhoud *et al.*, 1980) and *Galleria*

bioassays (Johnson and Hill, 1973b) on *Schistocerca* and *Locusta*

confirmed that the high JH levels in the early fourth instar

stadium decreased at the end of the stadium and were almost

undetectable during much of the fifth instar stadium and early

adulthood.

(b) Critical Timing

The sensitivity of various acridid tissues to JH-induced

effects appears to vary according to the development stage. In

Locusta and *Schistocerca*, exogenous JHA applied in small doses at

the beginning of the last larval instar cause changes in

morphology whereas the same doses applied later in the same instar

result in phase change effects (Nemec, 1970). In terms of the

effects of JH on wing and flight muscle development, several

authors (Staal, 1975; McCaffery and Page, 1978; Chapman *et al.*,

1978) believe the last nymphal instar to be the critical period.

Doane (1973), however, states that in locusts "...*the sensitive*

period for hormonal action in the case of...wing response was

during the post-molt period of rapid mitosis". In further
experiments by Beenakkers (1973), allatectomy failed to affect
locust flight muscle development. However, since the operation was
performed one day after adult ecdysis, the procedure may have been
too late to reverse a commitment and increase flight muscle dry
weight and protein content. The JH sensitivity of the system during
adult development and the length of the sensitive period have not
been extensively investigated. Rankin (1980) agrees that *"...more
work needs to be done to produce a dose-response curve of JH to
flight as well as JH titre determinations on reproductive males
and females".*

G. _JH Effects on Migration_

In the tropical grasshopper, *Z. variegatus,* wing length
is highly correlated with migratory capacity (McCaffery and Page, 1978),
and short-winged insects have poorly developed flight muscles
(Chapman *et al.,* 1978). JHA applications to final nymphal instar
grasshoppers resulted in progressively shorter wings as JH dosage
increased (McCaffery and Page, 1978). Chapman *et al.* (1978) state
that in determining the migratory status of these grasshoppers, *"the
important dimorphism is in the development of the wing muscles".*
Perhaps the high JH and ecdysone levels at the critical final nymphal
instar stage inhibit flight muscle as well as wing development in
solitaria locusts. Unfavorable JH levels for maximum flight muscle

development could be a major cause of non-migration in many insects.

If migrating insects possessed low JH levels in early adulthood, flight muscle development would be favored and reproductive development delayed. These characteristics are indeed seen in *gregaria* locust females. There may be a different response to JH of flight muscles and gonads in the two sexes during development, but this possibility has not yet been thoroughly investigated.

H. *Altering JH Levels with Precocene*

Recently, a new insect growth regulator precocene II has been discovered which has an anti-allatotropic effect (Bowers *et al.*, 1976). Actions of the compound on various insects include a reduction of CA activity, CA degeneration, precocious metamorphosis, and sterility (Bowers and Martinez-Pardo, 1977; Kruse Pedersen, 1978; Pener *et al.*, 1978; Schooneveld, 1979; Chenevert *et al.*, 1979). *In vitro* experiments have shown that precocene II is capable of directly inactivating the CA (Pratt and Bowers, 1977; Muller *et al.*, 1979). CA of *Oncopeltus*, excised and incubated *in vitro* with precocene, lost the ability to induce supernumerary molting which normally results in many CA-implanted insects (Muller *et al.*, 1979). This work has been supported by further *in vitro* experiments on *Schistocerca*. Precocene caused CA parenchyma cell degeneration in second, third, and fourth instar *Schistocerca* nymphs (Unnithan *et al.*, 1980). Bowers

and Aldrich (1980) confirmed that the brain and neurosecretion are not involved in precocene inactivation of the CA.

No side effects of precocene have yet been demonstrated. Non-target tissues such as the fat body and gut rapidly metabolize precocene (Pratt *et al.*, 1980). The authors suggest that this process may form the basis of precocene resistance in non-target tissues. Precocene sensitivity of the CA also varies from species to species. Of nine insect species tested, Ohta *et al.* (1977) found *"a variation of at least 37-fold in metabolic rate"* of precocene. The authors proposed that in insects in which precocene is rapidly degraded, the compound is less bioactive, and that this may be a basis for its selectivity.

Precocene applications are only effective at certain developmental stages. Kelly and Fuchs (1978) found no antigonadotropic effects on the adult female mosquito, *Aedes aegypti*, when precocene was applied one hour after adult emergence, while later applications after a blood meal slowed ovarian maturation and produced abnormal oviposition. Unnithan and Nair (1979) applied precocene II to fourth and fifth instar *Oncopeltus fasciatus* and found antiallatotropic effects in the earlier instars but not during the final instar. They proposed that *"apparently precocene is effective only when the insect's CA is active or it is free from any inhibitory control"*. Timing of precocene application is critical in *Locusta* as well (Kruse Pedersen, 1978).

Although the degeneration of precocene-treated CA is
permanent, some precocene effects seem to be short-lived. Sahota
and Farris (1980) temporarily arrested the normal degeneration of
flight muscles in log-colonizing female spruce bark bettles (*Dendroctonus
rufipennis*) by applications of precocene to adults. The normal
increase in flight muscle DNA/protein ratio was delayed. According
to Sahota and Farris (1980), *"...it would appear that with JH-induced
muscle degeneration, precocene II interferes with JH (production or
effects) and indirectly sustains transcription at a normal level"*.
The authors were not able to explain why muscle degeneration later
proceeded after a delay of 8 days. Comparable effects have been
observed in *Oncopeltus* (Masner *et al.*, 1979).

I. *Summary of Major Objectives*

The main purpose of this study was to investigate the role
of JH on the development of selected tissues in *M. sanguinipes* from
the following aspects:

1. What is the normal pattern of growth during early adulthood in
    male and female *M. sanguinipes*? What is the normal change in
    flight muscle protein content during this period?

2. How is the normal developmental pattern changed by topical JHA
    treatments? Is timing of JHA application critical even within
    an instar?

3. How is normal development changed when the activity of the CA is impaired by precocene II?

4. Can the developmental changes caused by the application of precocene II be reversed by subsequent JHA treatment?

MATERIALS AND METHODS

A. Rearing Techniques

The insects used in this study were of the non-diapause strain of *M. sanguinipes* (Pickford and Randell, 1969). Stock colonies were reared under crowded conditions (ca. 30 to 100 grasshoppers) in cages described by Pickford (1958) (Plate 1). Photoperiod was maintained at 12L:12D and temperatures at $30 \pm 5^{\circ}C$. The stock colonies and experimental insects were fed an alfalfa meal mixture (100 g alfalfa meal:100 g bran:10 g brewer's yeast:12 ml corn oil) supplemented by daily additions of fresh lettuce (Gillott and Dogra, 1972).

B. Growth Measurements

The initial experiments were aimed at establishing the normal growth patterns of adult males and females. Within 3 to 4 hrs after the imaginal molt, insects in the stock colony were transferred into glass jar cages (Plate 2). At various intervals after ecdysis, the grasshoppers were decapitated and weighed on a Mettler microbalance. The wing length, tibia length, and head width were measured with vernier calipers. Dissections were performed under a stereo-microscope. Using the methods described by Hill *et al.* (1968), the fat body, gonads, and flight muscles were removed, dried to constant weight at $35^{\circ}C$, and weighed on a microbalance. Flight muscles were later used for protein determinations. Analysis of variance was performed to determine the effects of age on adult body measurements.

PLATE 1: Cages used to rear stock colonies
of *M. sanguinipes*

PLATE 2: Cages used to determine the growth patterns of
normal, precocene- and juvenile-hormone-treated
grasshoppers

The significance of correlations between body parameters was also determined.

To obtain more precise estimates of daily changes in total body weight, the fresh weight was measured in the same insects during the first 9 days of adulthood. Since the presence of mature males affects the maturation rate of other female acridids (Uvarov, 1966), both sexes (2 males and 2 females) were placed in glass jars (Plate 2), and reared under conditions identical to those in the stock colony. Each day after the imaginal molt, the insects were anaesthetized with CO_2 and the fresh weight determined. The data were subjected to regression analysis to determine the overall relationship between age and fresh weight in both sexes. As distinct phases of somatic and reproductive development were evident in both sexes, additional regression analyses were performed on 1- to 3-day-old and 4- to 8-day-old insects.

C. *Protein Determinations*

The procedure employed to determine the protein content of the flight muscles was modified after Schacterle and Pollack (1973) and Lowry *et al.* (1951). Three pre-weighed portions of each sample (0.2-0.3 mg) were solubilized in 1.0 ml 0.5 N NaOH for 7 min at $100^{o}C$ (Lowry *et al.*, 1951). After adding 10 ml of copper reagent (10% sodium carbonate, 0.1% potassium tartrate, and 0.05% copper sulfate, but lacking NaOH), 4.0 ml of phenol reagent (Fisher

Scientific Co.) was added. Heating was omitted because the mixture was observed to be more stable at room temperature. After 10 min, transmittance readings were taken at 620 nm in a Spectronic 20. By knowing the total dry weight of the flight muscles and the amount used in each protein determination, the total protein content of the flight muscle was calculated.

D. *Chemical Treatments*

The role of JH in somatic and reproductive development was investigated using a juvenile hormone analogue (JHA) and an anti-allatotropin. Newly ecdysed fourth instars, fifth instars, and adults were removed from the stock colony. They were isolated in glass jars in groups of 6-10 grasshoppers/jar and exposed to conditions described previously (Plate 2). Adult emergence data were recorded for each insect. When adult emergence was asynchronous, the insects were marked individually with dots of Testor's PLA enamel paint (Testor Corp.).

(a) *JHA Studies*

At selected intervals, the insects were anaesthetized with nitrogen or carbon dioxide. As the JHA, R-20458 (6,7-epoxy-1-(p-ethylphenoxyl)-3,7-dimethyl-2-octene; Stauffer Chem. Co.), has been shown to be biologically active in *M. sanguinipes* (Elliott and Gillott, 1978), the compound was diluted appropriately. To determine the best solvent for the JHA trials, 1.0 µl samples

of acetone, olive oil, or an acetone/olive oil mixture (1:1) were applied using a micropipette to the abdomenal terga of six insects. The olive oil and acetone-oil mixture inhibited molting whereas acetone alone was non-inhibitory.

To determine the lethal and morphological effects of R-20458, the JHA was serially diluted in acetone. The JHA doses examined ranged from 0.375-0.0375 µg. One µl samples were applied topically with a micropipette to fourth and fifth instar nymphs. Treated insects were kept in glass jar cages until 4 to 5 days after adult emergence, when mortality and external appearance were noted.

The sensitivity of different stages of fifth instar nymphs to R-20458 was investigated by treating 1- to 6-day-old fifth instars with single applications of 0.05 µg JHA/insect. Five days after molting, the effects of the treatment on external morphology and growth of the various body tissues were assessed. Analysis of variance was performed on the data to see if there were any significant differences between various body measurements with different JHA application times. Correlations between body measurements were also determined.

In order to establish whether the effects of the JHA treatments on the various tissues were temporary or permanent, 0.05 µg R-20458 was applied to newly emerged fifth instar nymphs. The treated insects were dissected 5 and 14 days after adult emergence and their body measurements compared.

(b) *Anti-allatotropin Studies*

The insect anti-allatotropin, precocene II (6,7-dimethoxy-2,2-dimethyl chromene; Aldrich Chem. Co.) was stored under nitrogen at 6°C. Stock solutions were prepared by dilution in dimethyl sulfoxide (Pound and Oliver, 1979; Verma, 1981). Since 1 µl of the solvent exhibited no lethal or other deleterious properties (see Appendix 8), solvent-treated controls were not used in all the precocene experiments. Instead, the normal growth patterns established in the preceeding studies were used.

In order to determine a biologically-active but non-lethal dosage of precocene, fourth instar, fifth instar, and adult grasshoppers were treated with doses ranging from 25 to 1000 µg/insect. Effects on mortality and external appearance were recorded. To determine the effects of precocene II on adult body measurements, 1-day-old, fourth instar nymphs were treated with 300 µg precocene II. The insects were dissected 6-16 days after molting. To reverse the effects of precocene on molting, metamorphosis, and/or the development of the internal organs, insects which had been treated with precocene as newly ecdysed fourth instars were also treated with 0.05 µg R-20458. The JHA was applied at various intervals prior to the molt and 6 to 10 days after the molt.

E. *Statistical Analyses*

Analysis of variance, correlation matrices, and linear regression analysis were performed using the MIDAS computer package. [1]
(Michigan Interactive Data Analysis System); Fox and Guire, 1976.

RESULTS

A. *Normal Development*

The total body weight of males and females increased significantly during the first 9 days of adulthood (Table III). The daily increments are shown in Fig. 1. In both sexes, the fresh weight increased markedly during the first 3 days. However, after this, growth patterns were distinctly different. In males, the total body weight increased marginally to reach a maximum on day 5 when mating normally begins. Concurrent with this, the fresh weight declined and remained relatively stable for the remainder of the assessment period. In contrast, the total body weight of females increased significantly until day 8 when oviposition occurred.

In both sexes, no signficant change in tegmina and wing length, tibia length, or head width occurred after emergence (Table III, Fig. 2). However, significant changes in the dry weight of various internal organs were evident (Table III). In newly-emerged females, the ovary was poorly developed and weighed less than 2 mg (Fig. 3). The dry weight remained relatively constant until day 3, after which a pronounced increase occurred until day 8. At this point, the ovary contained mature eggs in the oviducts and weighed nearly 46 mg. The sharp decrease in ovarian dry weight reflected the deposition of these eggs. In newly emerged males, the testes were well developed. The mean combined dry weight of the testes-accessory gland complex was

Table IIi: Analysis of daily Changes in various growth parameters during early
adulthood in normal *M. sanguinipes*

Growth parameter	Males			Females		
	D.F.	F value	Significance	D.F.	F value	Significance
Total body fresh weight (mg)	8,44	3.49	.0034	7,39	5.92	.0001
Tibia length (mm)	8,44	1.65	.1373 NS	7,39	1.39	.2328 NS
Tegmina length (mm)*	8,44	0.31	.9581 NS	7,39	0.67	.6923 NS
Head width (mm)	8,44	1.05	.4178 NS	7,39	0.83	.5654 NS
Gonad dry weight (mg)	8,44	14.18	.0000	7,40	24.25	.0000
Fat body dry weight (mg)	8,44	2.41	.0298	7,40	6.87	.0000
Flight muscle dry weight (mg)	8,44	6.88	.0000	7,40	4.89	.0005

NS = not significant (P = 0.05)

* wing length comparable

/27
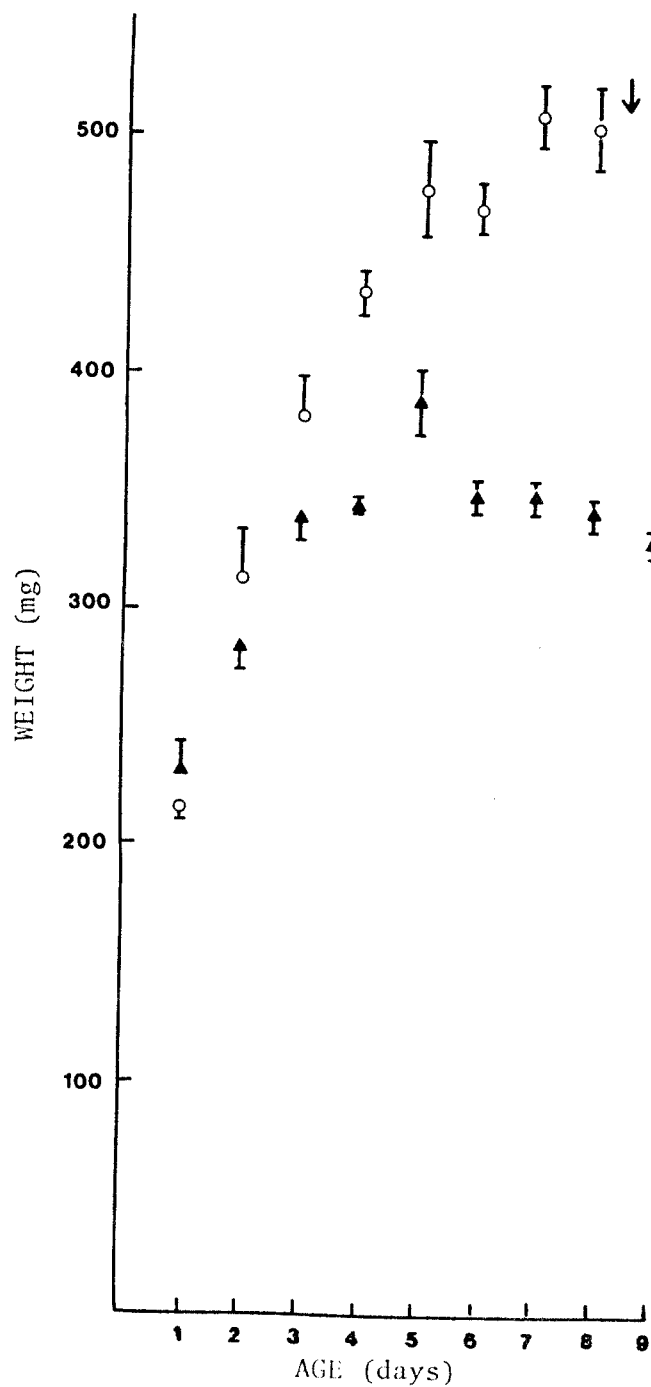
FIGURE 1: Changes in total body weight of normal males
(▲) and females (o) during early adulthood.
Arrow indicates approximate oviposition time.
In this and remaining figures, the mean ± S.E.
are indicated.
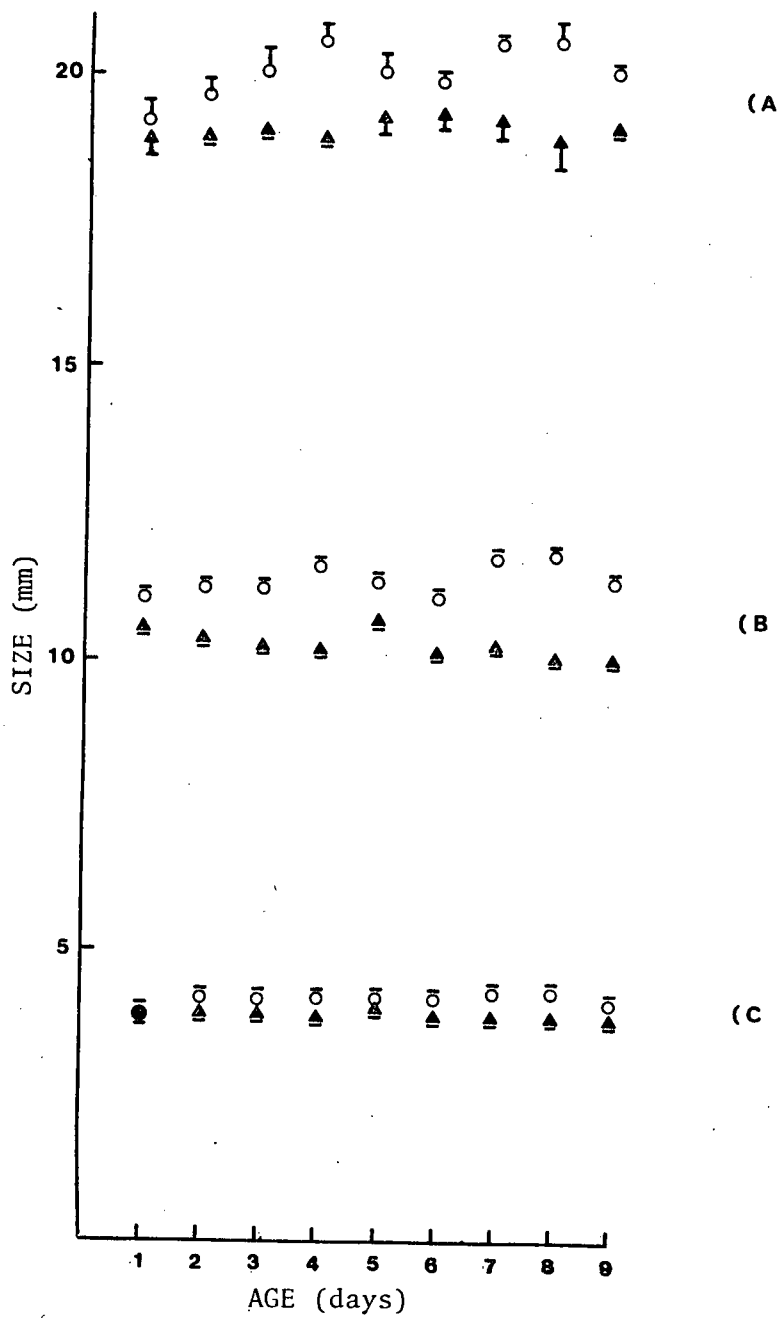
FIGURE 2: Changes in (a) tegmina and wing length, (b) tibia length, and (c) head width in normal males (▲) and females (o) during early adulthood.
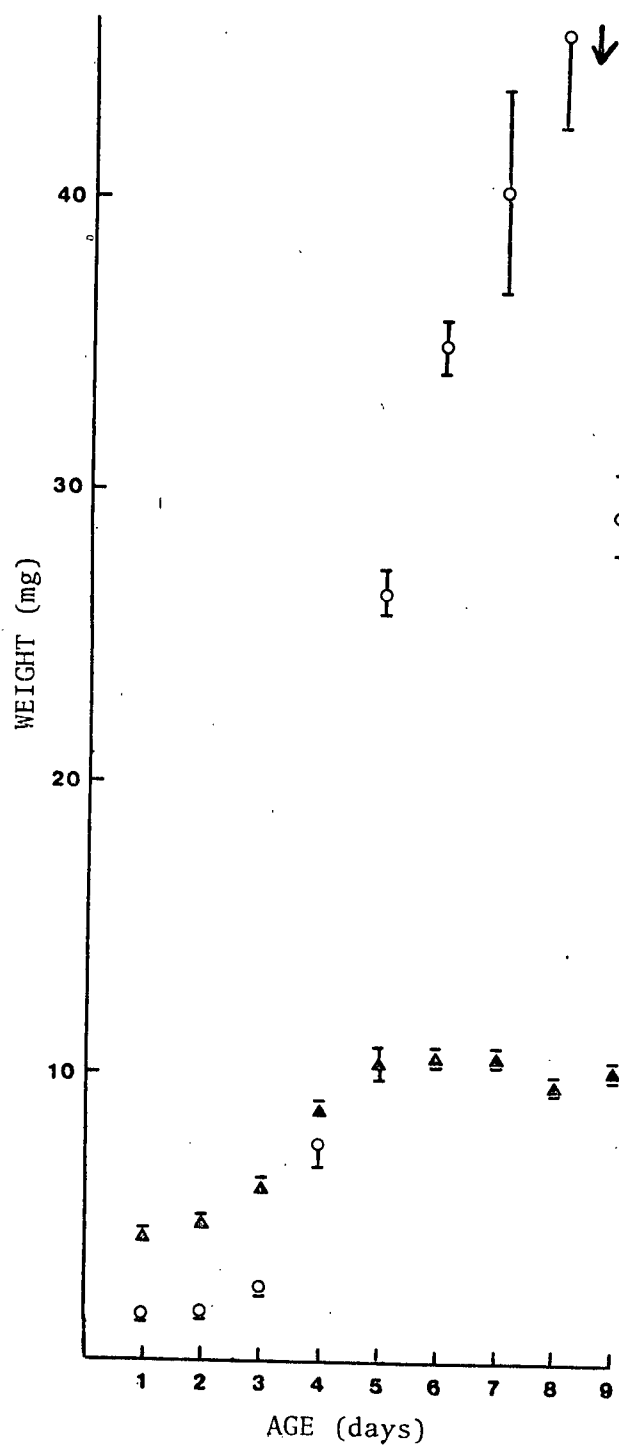
FIGURE 3: Changes in gonad dry weight of normal males (▲) and females (o) during early adulthood. Arrow indicates approximate oviposition time.

4.2 mg (Fig. 3). Pronounced weight increases occurred between days 3 and 5 when the dry weight of the complex stabilized at approximately 10 mg.

Highly significant (P = 0.01) changes in both fat body and flight muscle dry weight took place in males and females during early adulthood (Table III). Although the fat body was larger in females than in males, the pattern of daily changes in the dry weight of the tissue was similar in the two sexes (Fig. 4). In both, the fat body dry weight increased markedly after adult emergence and peaked on day 5, when mating usually begins. Then the dry weight decreased rapidly in both sexes, plateauing around days 8-9. Similarly, flight muscle dry weight was greater in females than in males (Fig. 5). However, in both sexes, the dry weight of the flight muscles followed a pattern similar to that of the fat body viz. rising sharply after adult emergence, peaking on days 5-6, then declining.

All body measurements were highly correlated with each other in normal *M. sanguinipes* (Fig. 6). The exception was the gonads, which were not correlated with tibia length or head width in either sex, nor with tegmina and wing length in females. Flight muscle dry weight was highly correlated with the other body measurements in normal *M. sanguinipes* (Table IV). Although the former fluctuated after imaginal ecdysis, it was still correlated with tegmina and wing length which are of fixed size after adult emergence.

More detailed fresh weight analyses were performed on a second group of newly emerged adults. The same insects, reared under
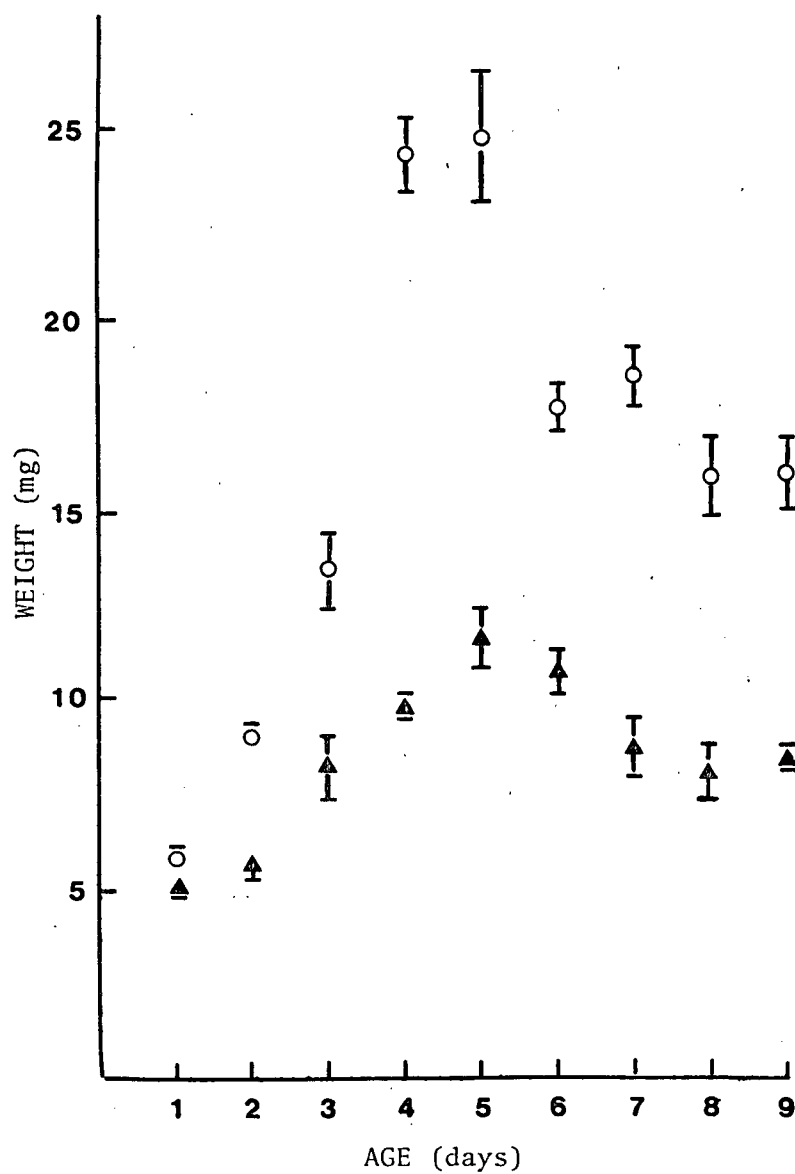
FIGURE 4: Changes in fat body dry weight of normal males (▲) and females (o) during early adulthood.
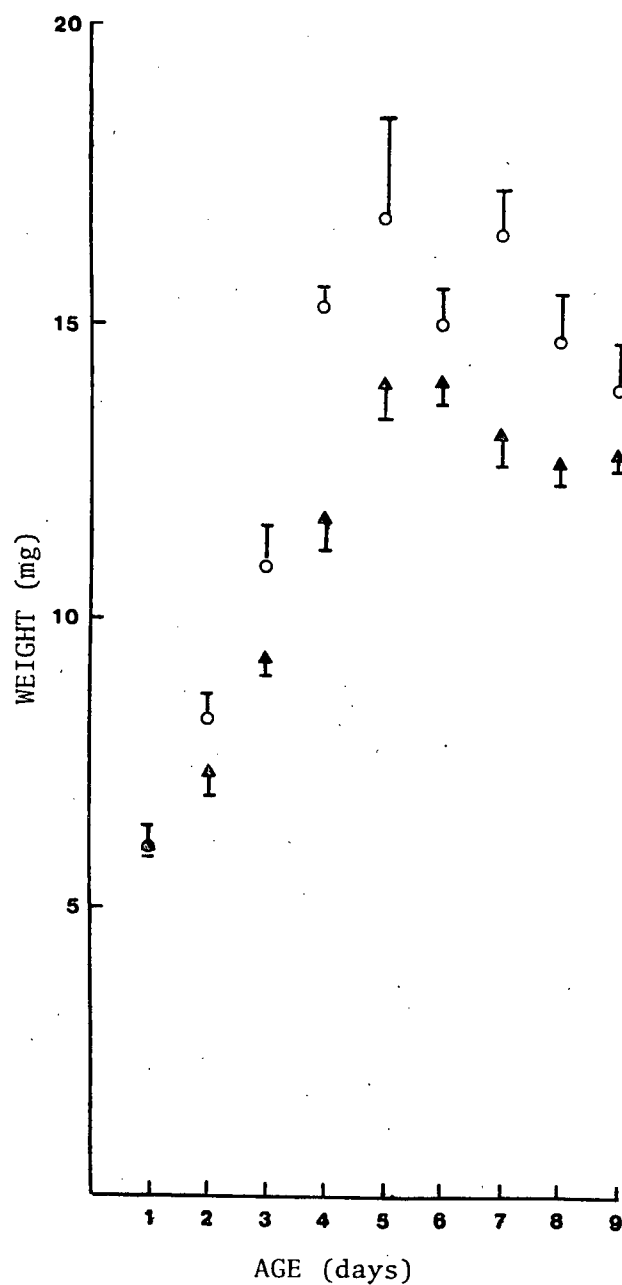
FIGURE 5: Changes in flight muscle dry weight of
normal males (▲) and females (o) during
early adulthood.
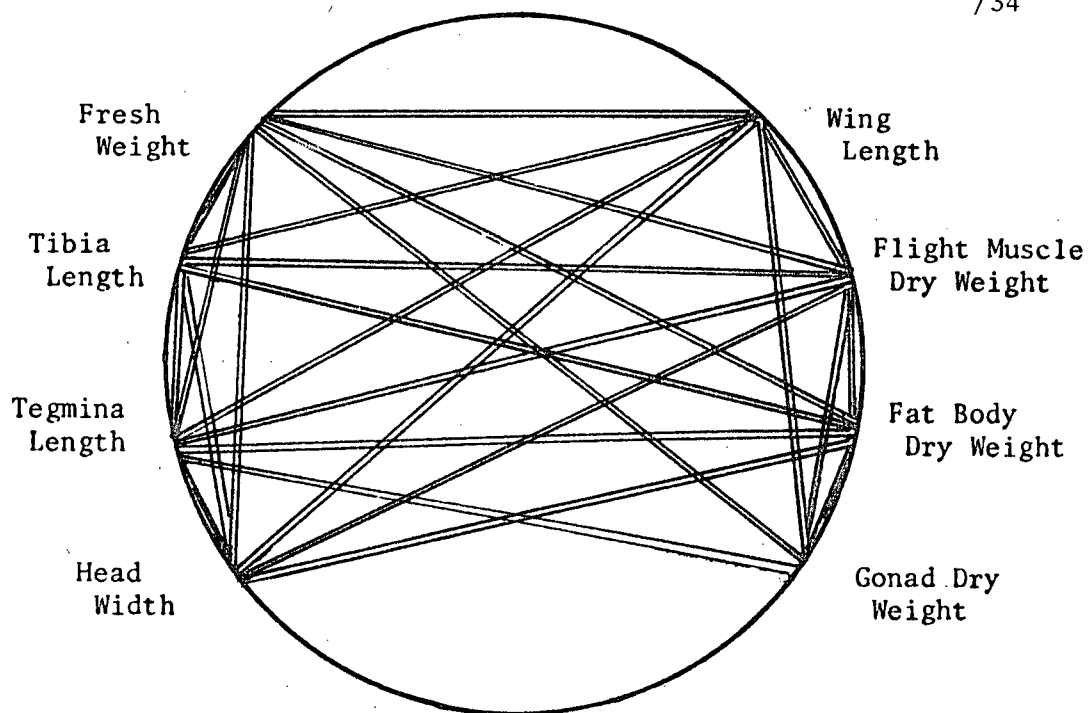
a) Males
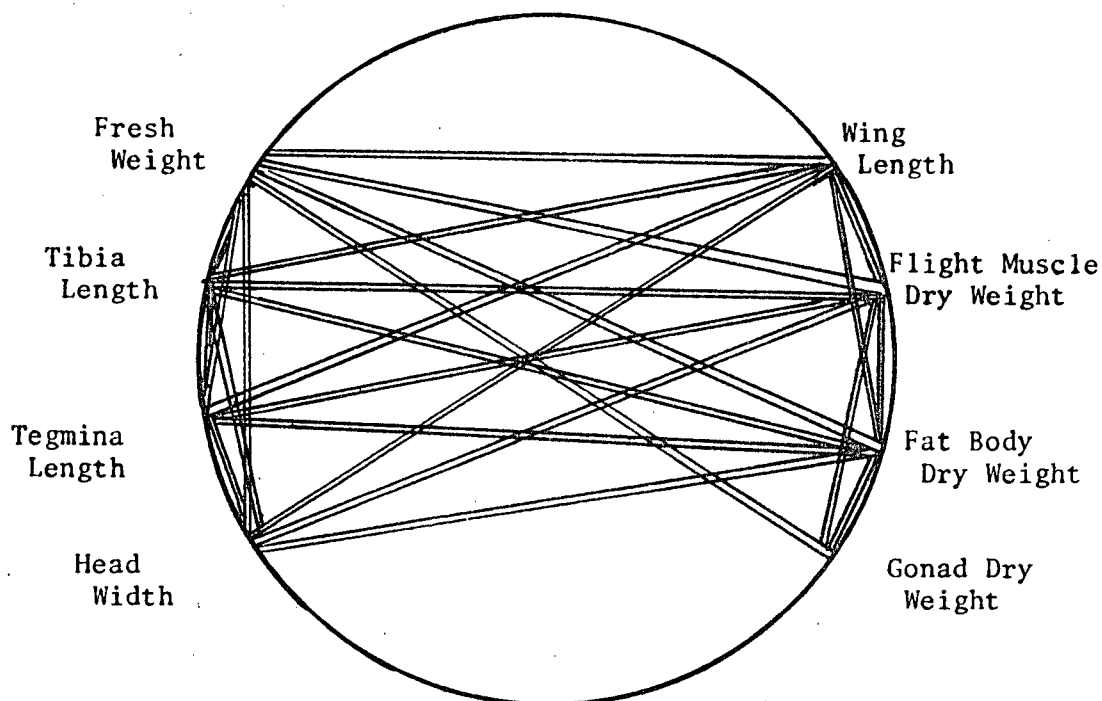
b) Females

FIGURE 6: Correlations among body measurements in normal adult
M. sanguinipes. In this and remaining figures, a
double line joining two points denotes a significant
correlation at the 1% level; a single line denotes
significance at the 5% level. When the growth of
two body parameters was not correlated, they were
not joined with a line. There were on negative
correlations.

TABLE IV: Correlation between the dry weight of the flight muscles and other
body measurements in normal *M. sanguinipes*

Growth parameter correlated with flight muscle dry weight	Males			Females		
	D.F.	R	Significance	D.F.	R	Significance
Total body fresh weight (mg)	54	.86	1%	54	.86	1%
Tibia length (mm)	54	.48	1%	54	.50	1%
Tegmina length (mm)	54	.55	1%	54	.56	1%
Wing length (mm)	54	.55	1%	54	.56	1%
Head width (mm)	54	.42	1%	54	.56	1%
Gonad dry weight (mg)	54	.85	1%	54	.56	1%
Fat body dry weight (mg)	54	.71	1%	54	.77	1%

density-controlled conditions, were used throughout this study.

Analysis of variance revealed a significant variation in fresh body

weight of both sexes during early adulthood (Table V). The increments

in fresh weight were highly dependent upon age, yielding significant

regressions in males and females for the 9-day period (Table V;

Fig. 7). Analysis of the residuals confirmed the normal distribution

of residual sizes, and the random distribution of the residuals

with respect to age. Regression equations of Y_{female} = 275.7 + 21.1X

and Y_{male} = 242.4 + 9.8X show a faster growth rate for females than for

males.

Examination of changes in the fresh weight and dry weight

of the internal organs in normal adults indicated that there were

two growth periods occurring in grasshoppers during the first 9 days

of adulthood (Figs. 1-5). During the first 3 days, limited reproductive

growth occurred in both sexes so the increase in fresh weight was due

primarily to growth of somatic tissues including the fat body, flight

muscles and cuticle. However, after this the converse was true so

that the increase in total body weight was largely due to the growth

of the reproductive organs. Fig. 8 shows the dependence of fresh

body weight on age during the two periods of early adult growth.

During the first 3 days, the slopes of the regression lines for males

and females were similar, being 28.8 and 26.3, respectively (Fig. 8).

Dependency of fresh body weight on age was significant in both sexes

at this time (Table V). However, after day 3, the regression equations

TABLE V: Linear regression analyses showing relationship between fresh weight (Y) and age (X) in young adult *M. sanguinipes*

Sex	Growth period	D.F.	F value	Significance	Regression equation
Males	Days 1 - 9	6,21	4.6	.0041	Y = 242.4 + 9.8 X
	Days 1 - 3	1,10	6.3	.0305	Y = 224.0 + 23.8 X
	Days 4 - 9	1,14	1.3	.2777 NS	Y = 273.9 + 4.6 X
Females	Days 1 - 9	6,21	22.3	.0000	Y = 275.7 + 21.1 X
	Days 1 - 3	1,10	29.5	.0003	Y = 264.4 + 26.3 X
	Days 4 - 9	1,14	16.2	.0013	Y = 310.4 + 15.6 X
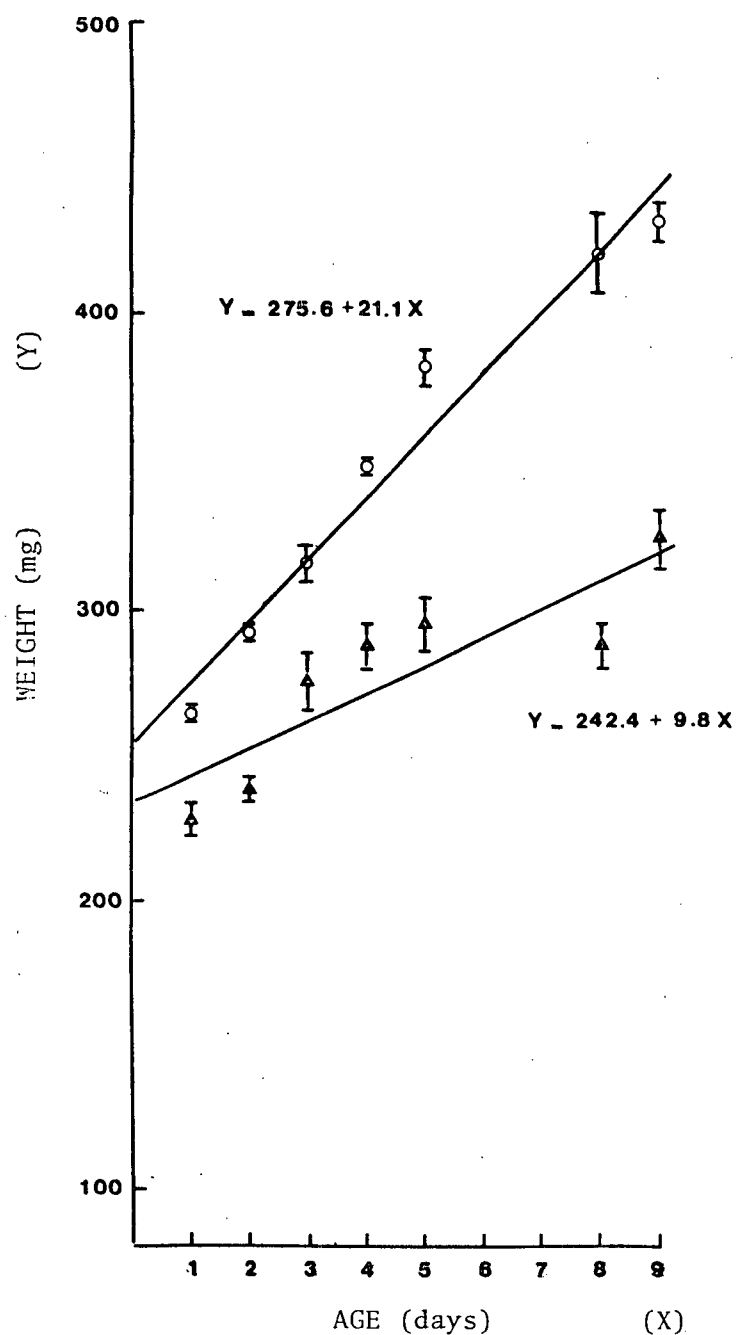
FIGURE 7: Regression lines showing relationship
between fresh body weight and age in
normal males and females during early
adulthood. Mean fresh weights of
males (▲) and females (o) with standard
errors (vertical lines) are also shown.
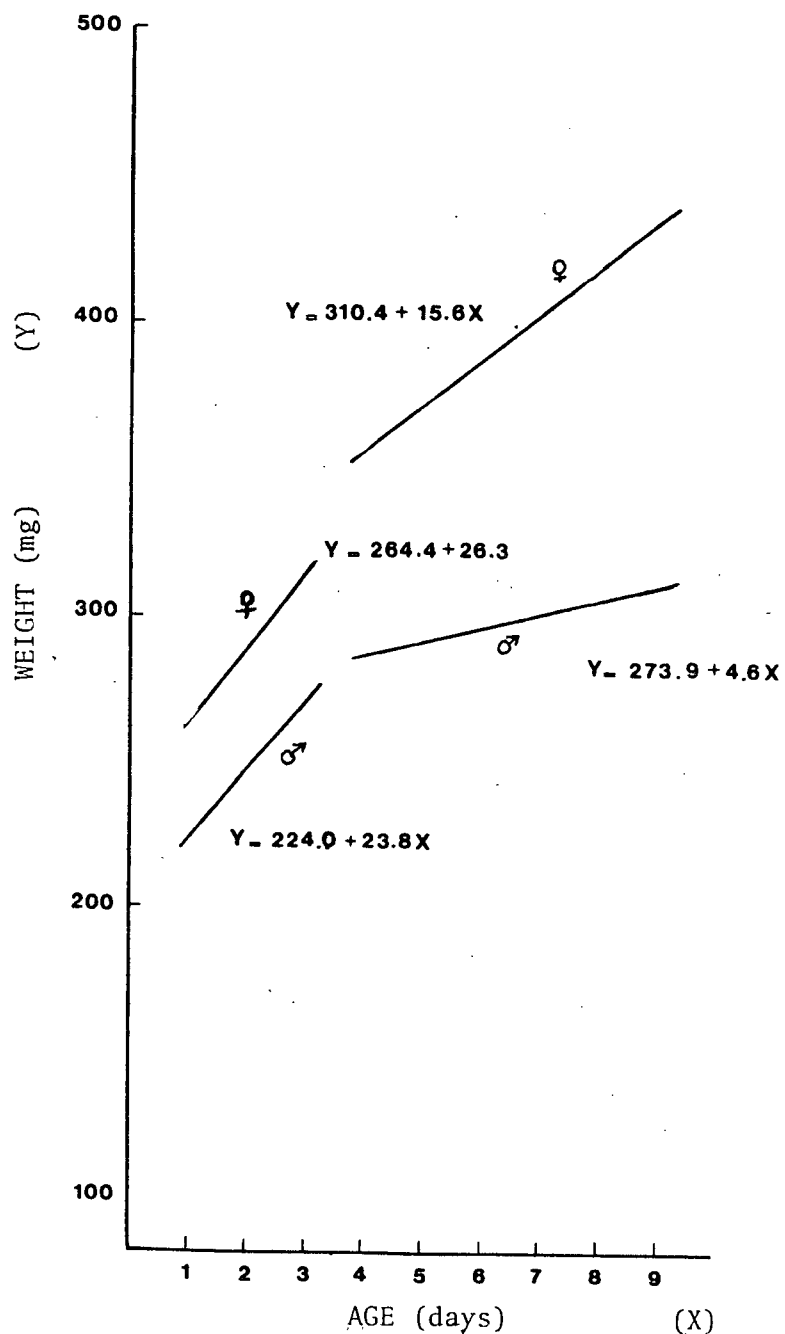
FIGURE 8: Regression lines showing relationships
between age and body fresh weight in
normal males and females during
the first 3 days of adulthood, and from
4 to 9 days after emergence.

for both sexes were quite different. In males, there was no significant change in fresh body weight with age (Table V); the slope of the regression line being only 4.6 (Fig. 8). However, in females, fresh body weight increased significantly with age (Table V) and yielded a regression slope of 15.6.

B. *Normal Flight Muscle Protein Content*

With the exception of Trial 1, flight muscle dry weight and protein content usually varied significantly ($P = 0.05$) with age in normal males and females (Table VI). In males, changes in the protein content and dry weight of the flight muscles paralleled each other (Fig. 9). In both trials, these two parameters increased rapidly immediately after ecdysis until days 5-7, when they decreased. Similar results were also observed in females (Fig. 10). Although the protein content and dry weight of the flight muscles were highly correlated, it is apparent that smaller muscles contained more protein per mg dry weight than the larger muscles (Table VII; Fig. 11). The slopes of the regression lines of these two parameters were similar for the two sexes in both trials. In trial 1, the regression slopes for males and females were 0.54 and 0.46, respectively, whereas in trial 2 they were 0.62 and 0.58. Analysis of the residuals confirmed the normal distribution of residuals with respect to age.

TABLE VI: Analysis of variance for flight muscle dry weight and
protein content during the first 9 days of adulthood

	Males				Females			
Tissue parameter	Trial	D.F.	F value	Significance	Trial	D.F.	F value	Significance
Flight muscle dry weight	1	7,15	2.34	.0793 NS	1	7,16	2,58	.0554 NS
	2	8,18	11.96	.0000	2	8,18	7.06	.0003
Flight muscle protein content	1	7,14	2.32	.0858 NS	1	7,15	4.38	.0079
	2	8,18	13.36	.0000	2	8,18	6.01	.0008

NS = not significant (P = 0.05)
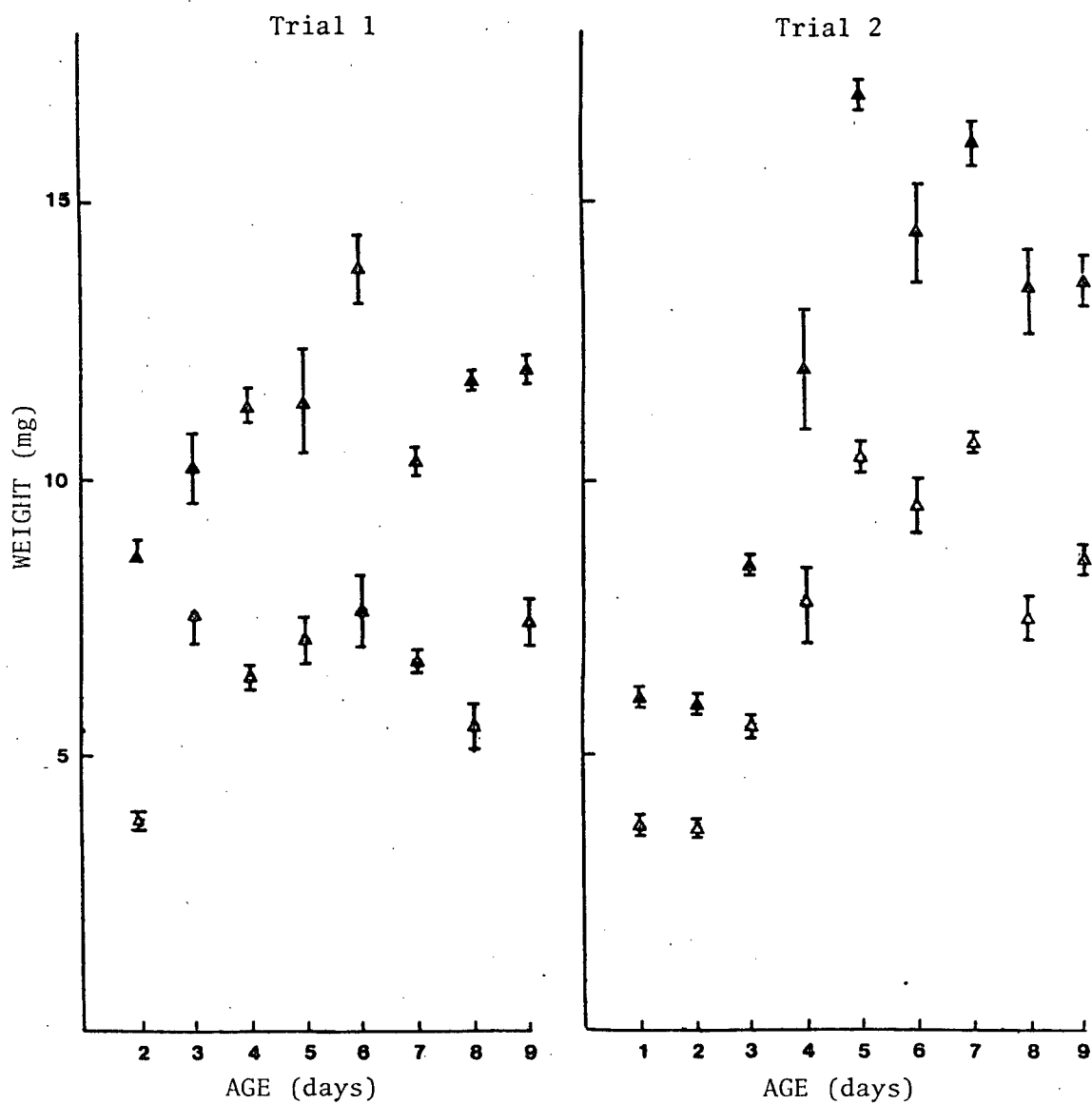
FIGURE 9: Changes in dry weight (▲) and protein content (Δ) of flight muscles in normal males during early adulthood.
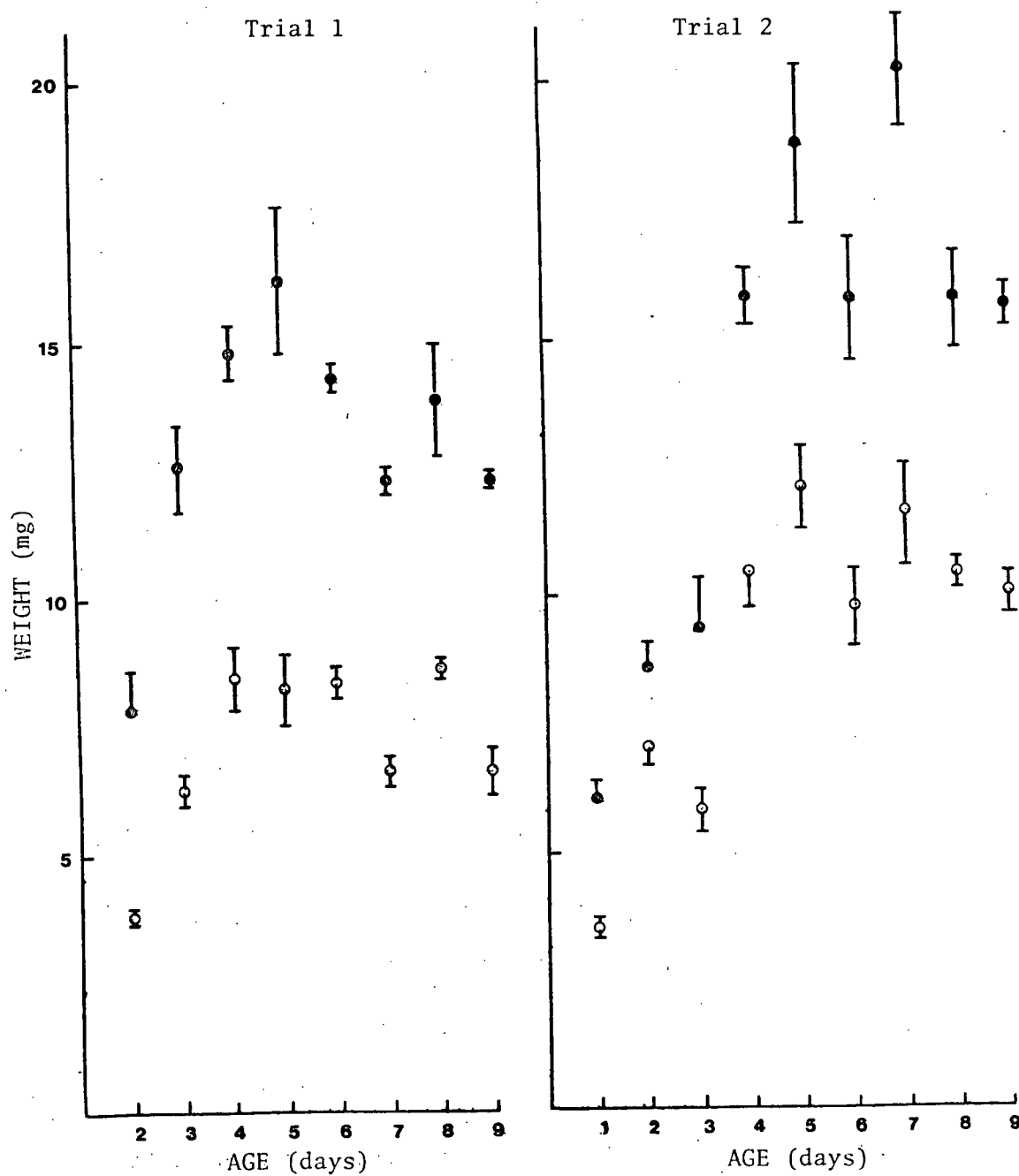
FIGURE 10: Changes in dry weight (●) and protein content (o) of flight muscles in normal females during early adulthood. For significance of daily changes in dry weight see Appendix 3.

TABLE VII: Linear regression equations showing relationship between protein content (Y) and dry weight (X) of the flight muscles during the first 9 days of adulthood

Sex	Trial	D.F.	F value	Significance	Regression equation
Males	1	1,20	13.89	.0013	Y = 0.46 + 0.54 X
	2	1,25	341.81	.0000	Y = 0.13 + 0.62 X
Females	1	1,21	39.60	.0000	Y = 1.05 + 0.46 X
	2	1,25	211.31	.0000	Y = 0.88 + 0.58 X
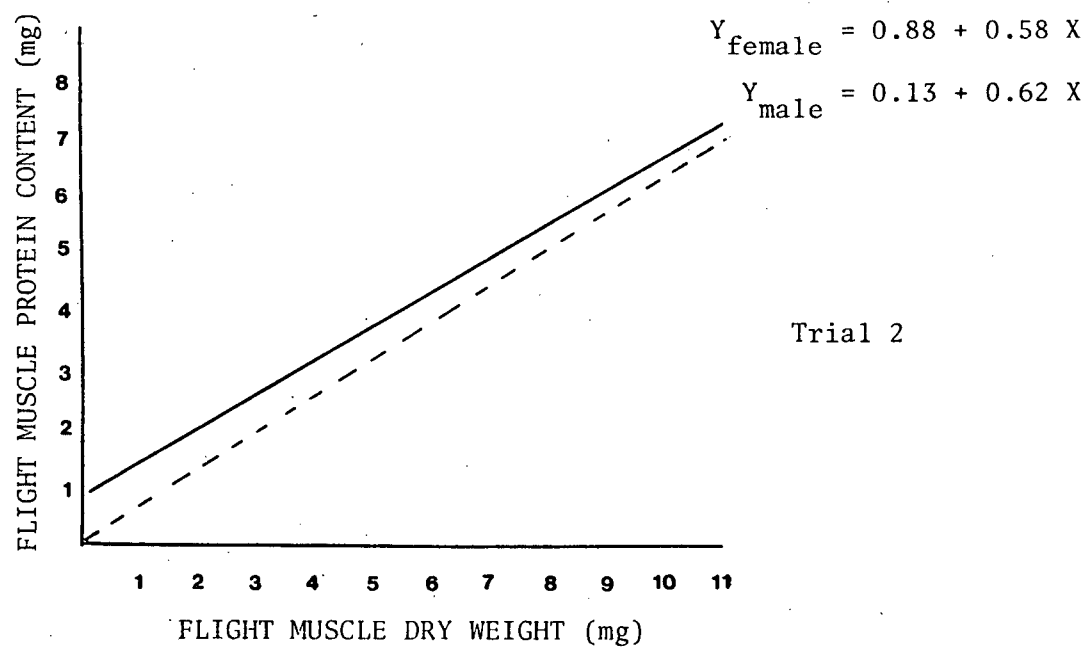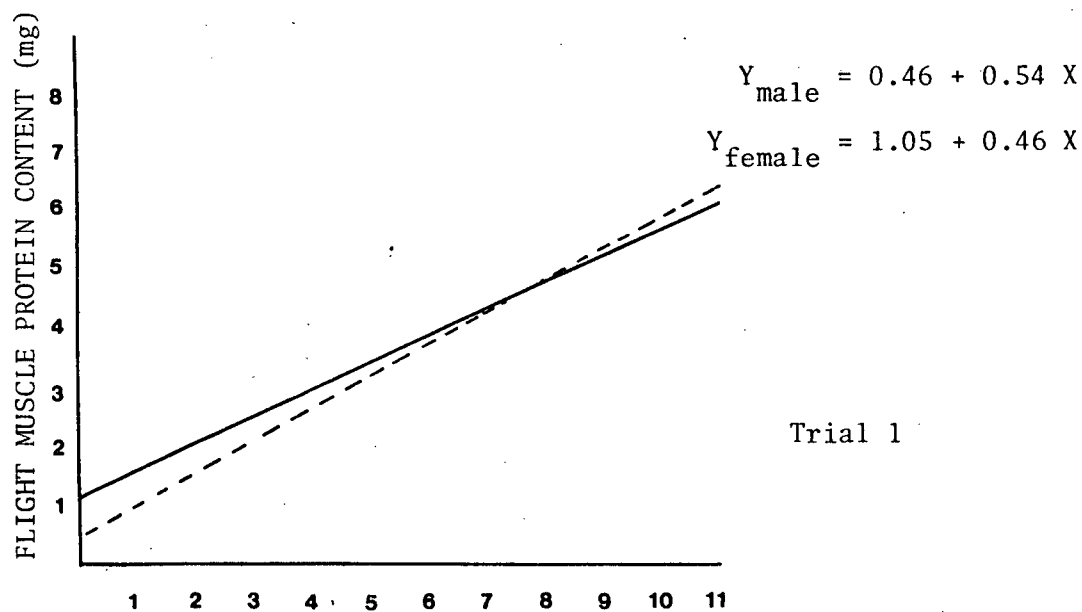
FIGURE 11: Regression lines showing the relationship between the protein content and the dry weight of flight muscles in normal males (----) and females (———) during early adulthood.

C. *JHA Studies*

(a) *Solvent Trials*

The sensitivity of fifth instar nymphs to topical application of 1 µl of three solvents is shown in Table VIII. Three days after application, both olive oil and a 1:1 mixture of olive oil/acetone resulted in delayed nymphal development and at least 50% mortality. In all instances, mortality occurred during the imaginal molt as the insects appeared unable to cast their exuvium. Because acetone exhibited no deleterious effects, it was chosen as the appropriate solvent in all subsequent JHA studies.

(b) *JHA Dose-Response Trials*

The sensitivity of different stages of *M. sanguinipes* to high dosages of R-20458 is shown in Table IXa. At higher doses (0.375 and 0.75 µg), the JHA killed most of the grasshoppers. Mortality was higher when R-20458 was applied to young and old fifth instars than when the treatment was applied during the middle of the stadium. Some of the surviving insects which had been treated with R-20458 in the middle of the fifth instar underwent a supernumerary molt to become large nymphal-adult intermediates with thick legs and varying color and wing length (Plates 3a and b). Sumpernumerary nymphs often died soon after the molt. Treated insects which retained the normal molting sequence often became adults with short and/or wrinkled wings. They were green or yellow in color, especially on the ventral portion of the abdomen.

TABLE VIII: Effects of topical application of three solvents
to fifth instar nymphs 3 days after treatment

Solvent	No. insects treated	No. surviving nymphs	No. surviving adults	No. died
olive oil	6	1	2	3
oil/acetone (1:1)	6	1	1	4
acetone	6	0	6	0

TABLE IXa: Sensitivity of different stages of *M. sanguinipes* to high dosages
of the JHA, R-20458

JHA conc.	Stage applied	No. insects treated	Mortality	No. normal adults	No. abnormal adults	Morphological and color effects
0.75 µg	ADULTS					
	< 2 h-old	3	3	0	0	no observable effects
	1 to 2 days-old	3	0	3	0	no observable effects
	5th INSTAR					
	late	5	4	N/A	N/A	no other effects observed
0.375 µg	4th INSTAR					
	early	6	6	0	0	no observable effects
	late	6	5	0	1	yellow color
	5th INSTAR					
	early	11	9	0	2	nymphal period prolonged; 1 very green
	middle	26	17	1	8	yellow or green; some with short wings; 5 insects underwent a supernumerary molt
	late	11	10	0	1	yellow color
	5th INSTAR					
	newly ecdysed to 60-h-old	11	11	0	0	no observable effects
	72-h-old	6	2	0	4	very green; 3 showed supernumerary molting
	80-h-old to 85-h-old	6	6	0	0	no observable effects
	95-h-old to 120-h-old	6	4	0	2	yellow-green color

PLATE 3a: Abnormally large female in supernumerary stadium (right)
resulting from JHA application (0.375 µg) to fifth
instar *M. sanguinipes*. For comparison, a normal
untreated female (left) is also shown.
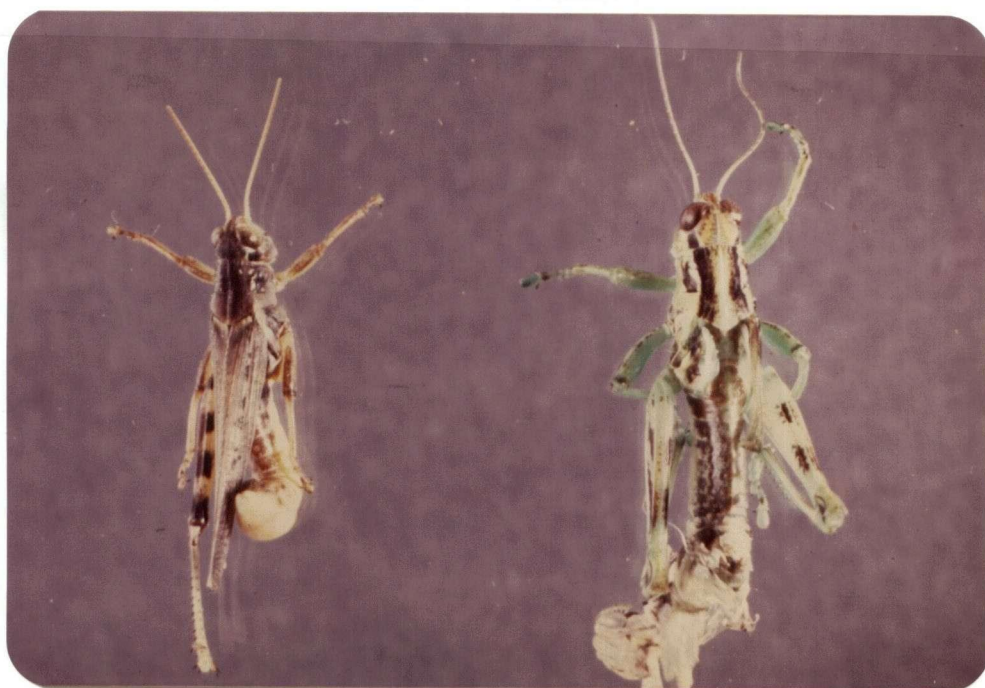
PLATE 3b: Normal, untreated male (left) and large, green,
short-winged supernumerary male (right) resulting from
JHA treatment described above. The treated insects
had difficulty casting their exuvia.

When a lower concentration (0.037 µg) was applied, mortality was infrequent and occurred only at the imaginal molt (Table IXb). Several normal-looking adults were produced, while others exhibited the characteristics observed at the higher dosages. An intermediate concentration of 0.075 µg JHA gave negligible mortality and a high frequency of easily observable JHA effects. To further minimize lethal effects, 0.05 µg R-20458 was applied in subsequent studies.

D. *Sensitivity of Fifth Instars to R-20458*

Single applications of 0.05 µg R-20458 to fifth instar insects of slightly varying ages resulted in dramatically different external and internal effects. JHA application to newly emerged fifth instar males and females produced mixed effects, ranging from insects which molted into normal-looking adults to those which became adultoids with pronounced nymphal characteristics. Juvenile characteristics exhibited by the latter included short, wings, reddish heads and pale cuticles marked with black (Plate 4a). A distinct, pale stripe extended the length of the pronotum. In normal adults, the pronotal stripe extends only halfway up the pronotum then blends into the beige-brown cuticle.

When the same dose of JHA was applied to 4-day-old fifth instar nymphs, the insects molted into normal-looking adults with long wings, beige-brown cuticle, and an indistinct pronotal stripe

TABLE IXb: Sensitivity of different stages of *M. sanguinipes* to low dosages of the JHA, R-20458

JHA conc.	Stage applied	No. insects treated	Mortality	No. normal adults	No. abnormal adults	Morphological and color effects
0.037 µg	5th INSTAR					
	misc.	12	1	N/A	N/A	many had shortened wings and yellow or green coloring; 1 underwent a supernumerary molt
	5-h-old	3	0	1	2	short wings
	20-h-old	2	0	0	2	wrinkled wings; one with short wings
	48-h-old	3	1	1	1	green color
	68-h-old	3	0	0	3	all slightly green
0.075 µg	5th INSTAR					
	mostly middle	15	1	0	14	green or yellow-green color; 4 underwent a supernumerary molt
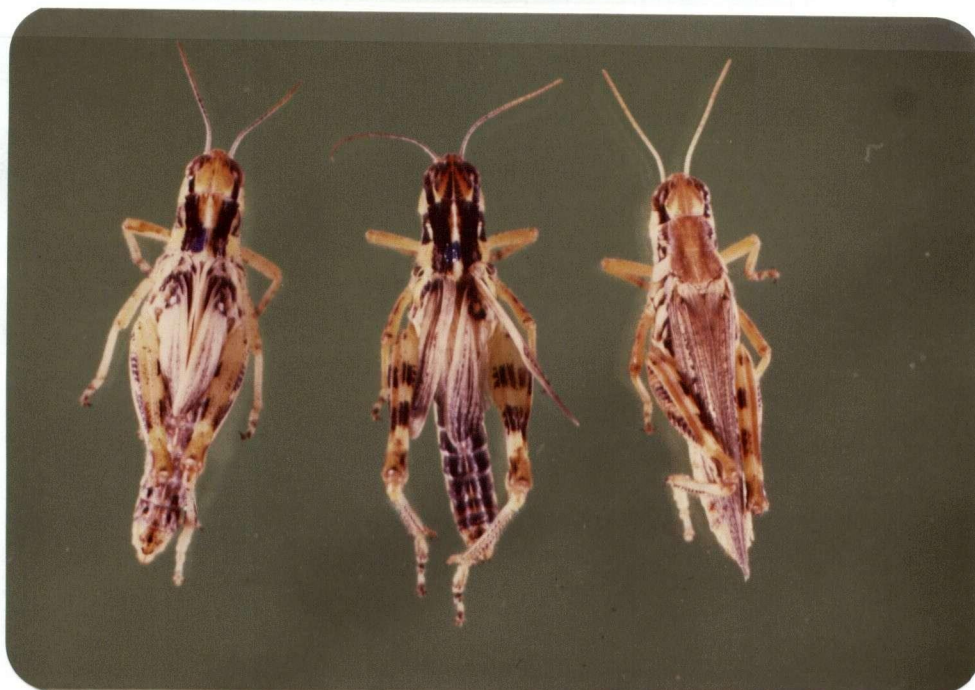
PLATE 4a: External morphology of 2-day-old adult females
that were treated with 0.05 μg R-20458 as newly
emerged fifth instar nymphs. An untreated
adult is shown on the far right.
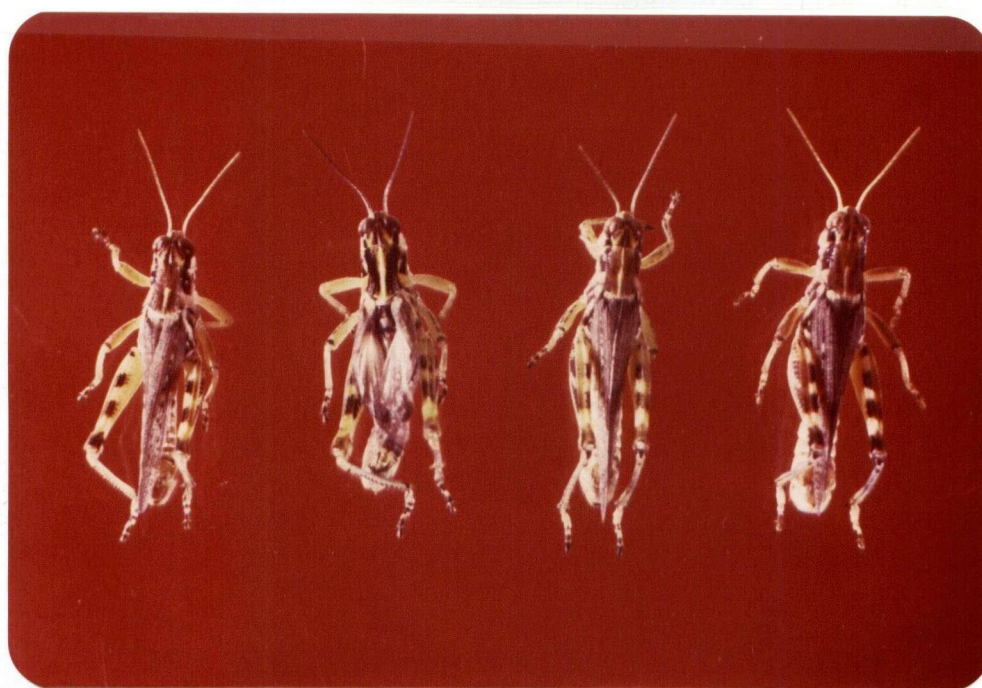
PLATE 4b: Two-day-old adult males showing the effect of a
single application of 0.05 μg R-20458 to (left
to right) 4-, 5-, and 6-day-old fifth instar
nymphs. An untreated grasshopper of comparable
age is shown on the far right.

(Plate 4a). Similar JH treatments applied to fifth instars from late day 4 to early day 5 usually resulted in adults with green-brown to bright green cuticles and slightly shortened to very short tegmina and wings (Plates 4b, 5). Maximum green color was usually seen around day 5 of adulthood. The distinct pale pronotal stripe observed in grasshoppers which received JHA at fifth instar emergence was also present in the short-winged, green insects. Although the tegmina and wings are of equal length in normal *M. sanguinipes*, treated insects often had shorter wings than tegmina. Furthermore, in some insects, the tegmen and wing were of normal length on one side of the body but shorter on the other. The same JHA dose applied to 5- or 6-day-old fifth instars produced little effect, although a faint green coloration and slight wing shortening occurred in some of the adults.

Tables Xa and b compare the body measurements of normal 5-day-old adults (average of two previous trials) and those that had been treated with 0.05 µg JHA during the fifth instar. In males, JHA application resulted in significant (P = 0.05) reductions in total fresh body weight, tegmina length, wing length, and flight muscle dry weight. Except in trial 2, the tegmina-wing length and flight muscle dry weight were significantly reduced in JHA-treated females. Both sexes experienced significant changes in gonad dry weight after JHA application in trial 1 but not in trial 2. Tibia lengths, head widths, and fat body dry weights were not significantly different from those of untreated insects.

PLATE 5:   Two-day-old adult males, showing the effect
          of a single application of 0.05 μg R-20458
          to   (a) 5-,   (b) 5-, and   (c) 6-day-old
          fifth instar nymphs. An untreated control
          (d) is also indicated.

TABLE Xa: Comparison of various body parameters of normal 5-day-old male adults and those treated with 0.05 μg R-20458 at various intervals during the fifth stadium*

Body parameter	Untreated Mean ± S.D.	JHA Trial 1				JHA Trial 2			
		Mean ± S.D.	D.F.	F value	Significance	Mean ± S.D.	D.F.	F value	Significance
Total body fresh weight (mg)	387.4±66.8	323.6±38.5	1,20	7.70	.0121	327.8±31.7	1,22	8.62	.0079
Tibia length (mm)	10.8±0.6	10.7±0.5	1,20	0.00	.9494 NS	10.6±0.5	1,20	0.15	.6988 NS
Tegmina length (mm)	19.3±1.6	15.2±3.5	1,20	7.72	.0120	15.1±3.9	1,22	6.47	.0189
Wing length (mm)	19.3±1.6	13.7±3.7	1,20	12.41	.0023	14.5±3.6	1,22	9.79	.0051
Head width (mm)	4.0±0.2	3.9±0.2	1,20	2.04	.1692 NS	3.8±0.1	1,22	5.78	.0255
Gonad dry weight (mg)	10.3±2.6	7.9±1.3	1,20	8.78	.0080	9.2±1.7	1,22	1.57	.2241 NS
Fat body dry weight (mg)	11.7±4.0	8.5±3.5	1,20	3.23	.0883 NS	9.5±2.6	1,22	2.28	.1460 NS
Flight muscle dry weight (mg)	14.1±3.7	9.8±2.0	1,20	12.20	.0024	9.8±2.5	1,22	10.41	.0040

*In Tables Xa-XIb, R-20458 was applied on days 1, 3, 4, 5 or 6 of the fifth stadium in Trial 1 whereas in Trial 2, the JHA was applied on days 1, 2, 4, 4 1/2, 4 3/4 or 5 of the fifth stadium.

TABLE Xb: Comparison of various body parameters of normal 5-day-old female adults and those treated with 0.05 µg R-20458 at various intervals during the fifth stadium*

Body parameter	Untreated Mean ± S.D.	JHA Trial 1 Mean ± S.D.	D.F.	F value	Significance	JHA Trial 2 Mean ± S.D.	D.F.	F value	Significance
Total body fresh weight (mg)	475.8±85.2	431.8±66.8	1,20	1.59	.2222 NS	448.6±47.0	1,20	0.90	.3555 NS
Tibia length (mm)	11.4±0.6	11.7±0.6	1,20	1.48	.2383 NS	11.7±0.4	1,19	2.01	.1734 NS
Tegmina length (mm)	20.0±1.6	16.0±3.6	1,20	6.69	.0181	17.5±2.9	1,20	3.78	.0667 NS
Wing length (mm)	20.0±1.6	15.0±3.7	1,20	9.71	.0057	16.4±3.1	1,20	7.02	.0158
Head width (mm)	4.2±0.2	4.2±0.2	1,20	0.07	.7880 NS	4.2±0.1	1,20	0.17	.6828 NS
Gonad dry weight (mg)	26.6±3.9	13.5±11.8	1,20	6.82	.0172	33.9±14.7	1,20	1.41	.2496 NS
Fat body dry weight (mg)	24.8±8.4	20.3±6.6	1,20	1.71	.2061 NS	18.8±6.7	1,20	2.94	.1028 NS
Flight muscle dry weight (mg)	17.5±4.8	12.0±2.7	1,20	11.07	.0024	12.0±1.8	1,20	15.02	.0010

Tables XIa and b show the effects of R-20458 on adult body measurements when 0.05 µg of the JHA was applied at various times during the fifth stadium. In general, the timing of JHA application only had a significant effect upon the fat body, tegmina, and wings of 5-day-old adults. In some cases significant changes in ovarian dry weight, tibia length, and flight muscle dry weight were also produced. However, these changes were not consistent enough to provide a basis for accurately predicting the size of these parameters from JHA application time (Appendix 4). Correlations among body parts were much lower in JHA-treated than in normal grasshoppers, and varied with sex and with trial (Figs. 12a and b).

E. *Effects of Adult Aging on JHA-Treated Insects*

Tables XIIa and b show the various body measurements in normal insects and those treated with R-20425 as newly emerged fifth instars. The measurements were taken 5 or 6 and 14 days after adult emergence. Treated insects had shorter than normal tegmina and wings in both age groups and female gonad dry weight was variably altered. Five- to 6-day-old males and females exhibited reduced flight muscle dry weight after fifth instar JHA treatment. However, no reduction in flight muscle dry weight was seen in 14-day-old adults after the same treatment. The main effect of aging on the body parameters was reduced fat body dry weight viz. in both sexes, the weight in 14-day-old insects was about half that of 5- to 6-day-old insects.

TABLE XIa: Overall effects of R-20458 on adult male body measurements when applied at various times during the fifth stadium

Body parameter	Trial 1			Trial 2		
	D.F.	F value	Significance	D.F.	F value	Significance
Total body fresh weight (mg)	4,14	2.86	.0808 NS	5,16	0.63	.6833 NS
Tibia length (mm)	4,14	5.07	.0170	5,16	0.69	.6423 NS
Tegmina length (mm)	4,14	23.12	.0000	5,16	25.23	.0000
Wing length (mm)	4,14	30.78	.0000	5,16	16.43	.0001
Head width (mm)	4,14	3.03	.0704 NS	5,16	0.37	.8561 NS
Gonad dry weight (mg)	4,14	1.31	.3313 NS	5,16	2.91	.0653 NS
Fat body dry weight (mg)	4,14	4.74	.0210	5,16	3,56	.0370
Flight muscle dry weight (mg)	4,14	4,65	.0222	5,16	1.14	.3974 NS

TABLE XIb: Overall effects of R-20458 on adult female body measurements when applied at various times during the fifth stadium

Body parameter	Trial 1			Trial 2		
	D.F.	F value	Significance	D.F.	F value	Significance
Total body fresh weight (mg)	4,14	2.25	.1365 NS	4,14	1.19	.3742 NS
Tibia length (mm)	4,14	6.60	.0072	4,12	3.08	.0744 NS
Tegmina length (mm)	4,14	5.76	.0114	4,14	14.27	.0004
Wing length (mm)	4,14	20.84	.0001	4,14	41.52	.0000
Head width (mm)	4,14	1.86	.1945 NS	4,14	0.21	.9267 NS
Gonad dry weight (mg)	4,14	111.66	.0000	4,14	1.97	.1749 NS
Fat body dry weight (mg)	4,14	5.08	.0170	4,14	16.67	.0002
Flight muscle dry weight (mg)	4,14	2.46	.1132 NS	4,14	3.77	.0403
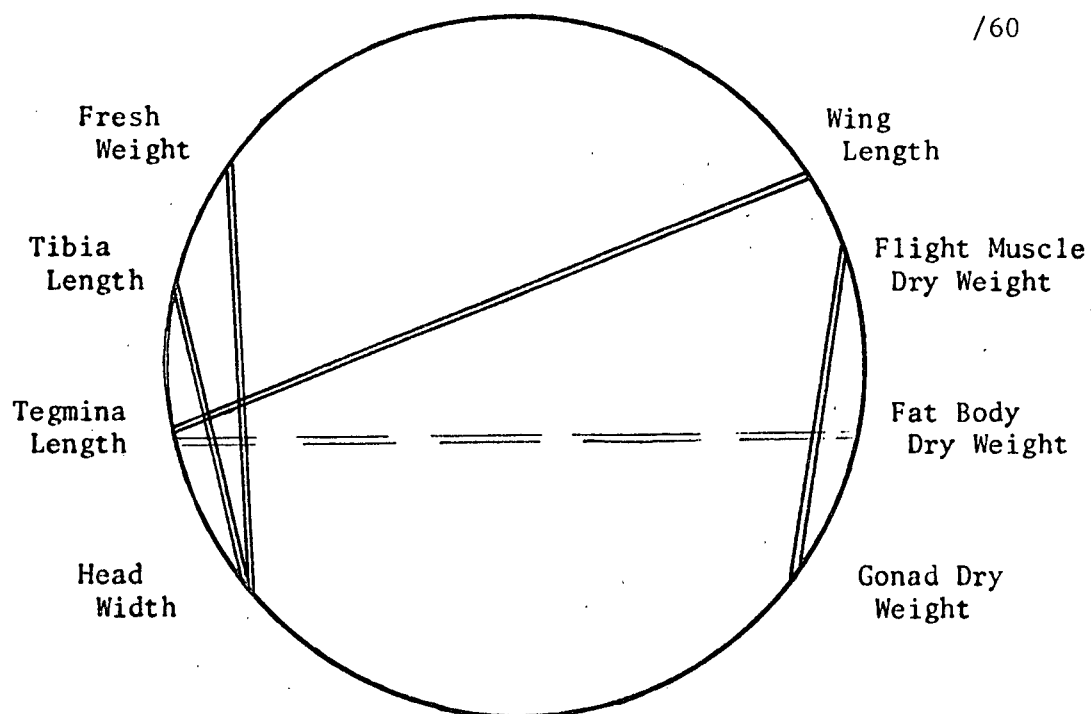
(i) Trial 1
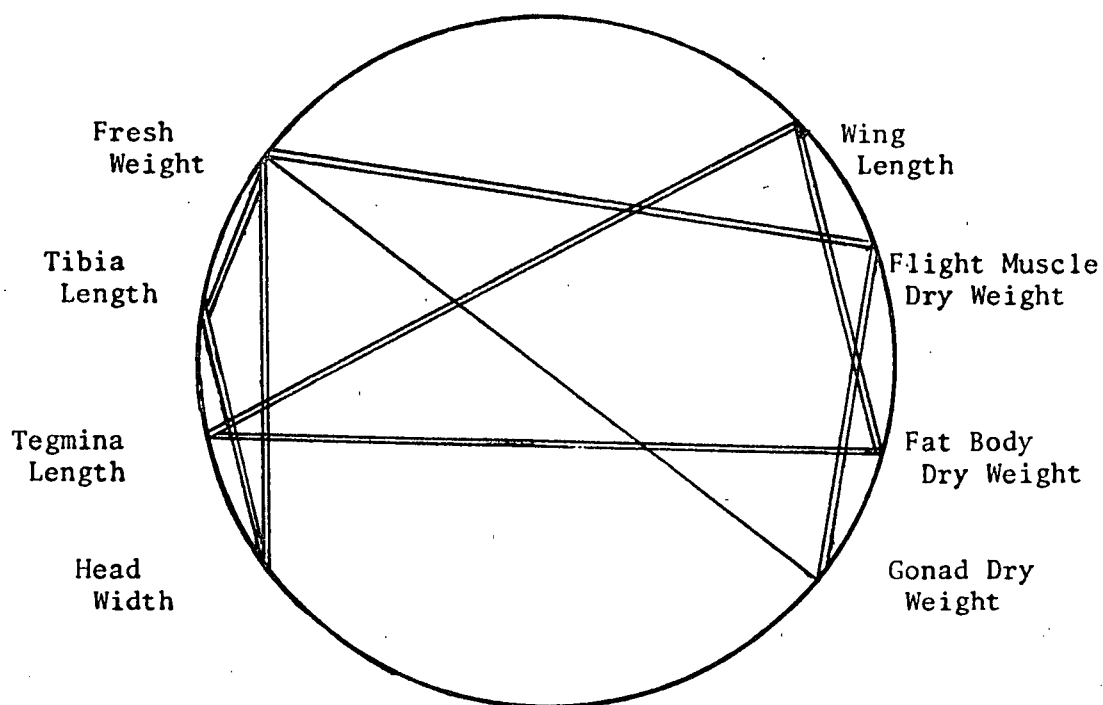
(ii) Trial 2

FIGURE 12a: Correlations among body measurements in male adults after R-20458 was applied during the fifth stadium. Negative correlations in this and succeeding figure are denoted by dotted lines.

Fresh
Weight

Tibia
Length

Tegmina
Length

Head
Width

Wing
Length

Flight Muscle
Dry Weight

Fat Body
Dry Weight

Gonad Dry
Weight

(i) Trial 1

Fresh
Weight

Tibia
Length

Tegmina
Length

Head
Width

Wing
Length

Flight Muscle
Dry Weight

Fat Body
Dry Weight

Gonad Dry
Weight
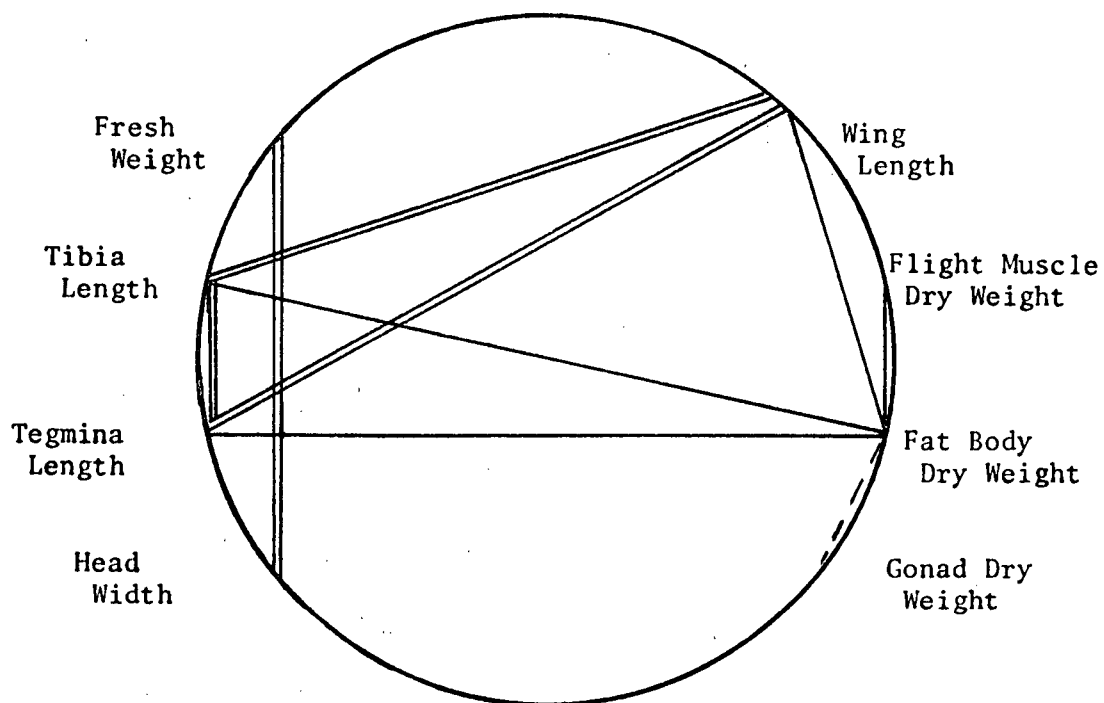
(ii) Trial 2

FIGURE 12b: Correlations among body measurements in female
adults after R-20458 was applied during the
fifth stadium.

TABLE XIIa: Mean body measurements (±S.D.) in untreated and JHA-treated males
dissected as 5- or 6-day-old adults, or 14-day-old adults,
(N = 3 to 6)

Body parameter	5- or 6-day-old adults		14-day-old adults	
	Untreated	JHA-treated	Untreated	JHA-treated
Total body fresh weight (mg)	387.4±66.8	361.2±30.5	344.8±40.1	318.6±22.7
Tibia length (mm)	10.8±0.6	11.1±0.5	10.7±1.0	10.6±0.0
Tegmina length (mm)	19.3±1.6	16.4±1.3	18.7±1.6	10.6±1.8
Wing length (mm)	19.3±1.6	11.8±1.0	18.7±1.6	10.6±1.8
Head width (mm)	4.0±0.2	4.0±0.1	3.9±0.2	3.9±0.1
Gonad dry weight (mg)	10.3±2.6	8.0±3.2	11.2±1.1	11.7±1.8
Fat body dry weight (mg)	11.7±4.0	9.7±0.6	5.8±1.0	5.7±1.5
Flight muscle dry weight (mg)	14.1±3.7	11.8±1.0	12.0±2.1	12.0±0.7

TABLE XIIb: Mean body measurements (±S.D.) in untreated and JHA-treated females dissected as 5- or 6-day-old adults, or 14-day-old adults (N = 3 to 6)

Body parameter	5- or 6-day-old adults		14-day-old adults	
	Untreated	JHA-treated	Untreated	JHA-treated
Total body fresh weight (mg)	475.8±85.2	383.7±80.3	427.4±92.0	420.1±56.7
Tibia length (mm)	11.4±0.6	11.4±0.1	11.6±0.8	11.1±0.6
Tegmina length (mm)	20.0±1.6	14.6±2.4	19.9±1.7	13.0±2.8
Wing length (mm)	20.0±1.6	12.6±1.6	19.9±1.7	11.9±1.6
Head width (mm)	4.2±0.2	4.1±0.2	4.2±0.3	4.2±0.2
Gonad dry weight (mg)	26.6±3.9	6.7±2.4	33.3±1.81	51.8±22.2
Fat body dry weight (mg)	24.8±8.4	19.9±7.0	9.2±4.6	7.1±2.0
Flight muscle dry weight (mg)	17.5±4.8	11.0±2.6	10.6±2.2	9.9±1.1

Correlations between body measurements were reduced with age and with JHA treatment in both sexes (Fig. 13a-d). In 13-day-old, JHA-treated males and females, the only significant correlation (P = 0.05) was between tegmina and wing length.

F. *Sensitivity of M. sanguinipes to Precocene II*

Table XIII shows the dose response of *M. sanguinipes* to precocene II applied at various times during the fourth, fifth, and adult instars. Precocious metamorphosis was produced in early fourth instars when doses of 200-300 μg precocene were applied (Plates 6a and b). Lower doses had no apparent effect on adult emergence while doses exceeding 400 μg produced high mortality (Table XIII). Two of the survivors of these high precocene doses exhibited JHA-like effects, namely short wings and juvenile coloration. Precocene II did not result in precocious metamorphosis when applied to fifth instars, although applications of 400-500 μg caused some mortality. In two cases, the ovaries of 5-day-old adult females remained undeveloped.

Newly-emerged adults experienced some mortality and growth retardation when 1000 μg precocene II was applied. At least 500 μg was needed for any effect on newly-emerged adults and ovarian development appeared to be normal. However, more extensive tests would be necessary to confirm this finding.
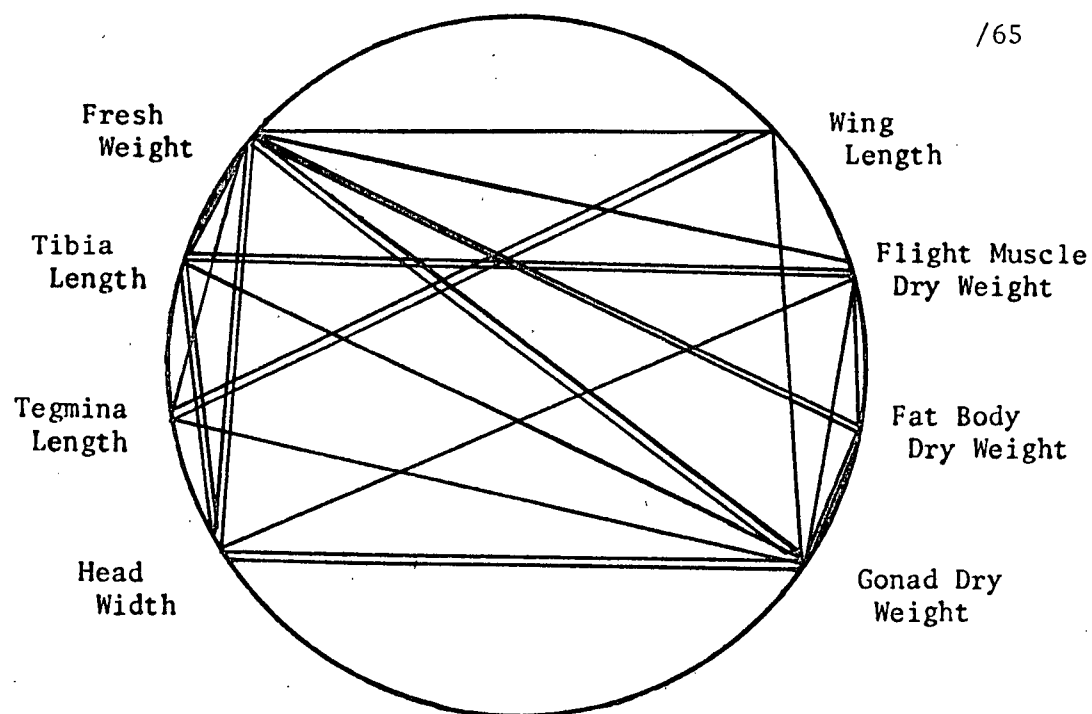
(i) Untreated insects
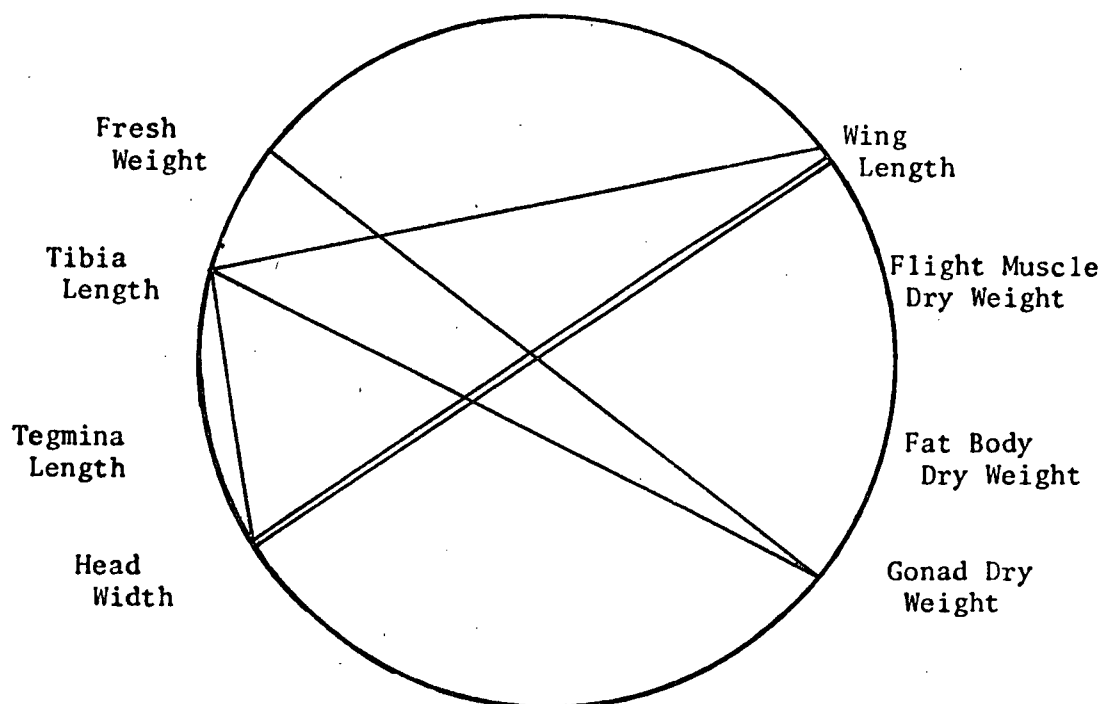
(ii) JHA-treated

FIGURE 13a: The effect of aging and JHA treatment on
             correlations among body measurements in 5-
             or 6-day-old adult males.
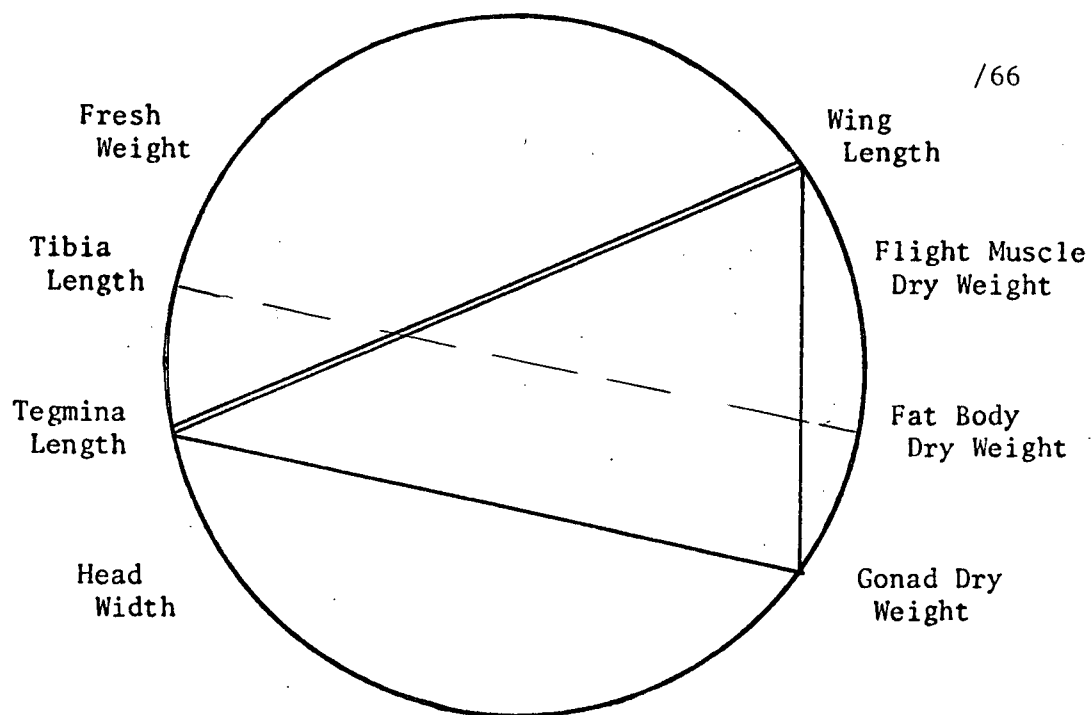
(i) Untreated
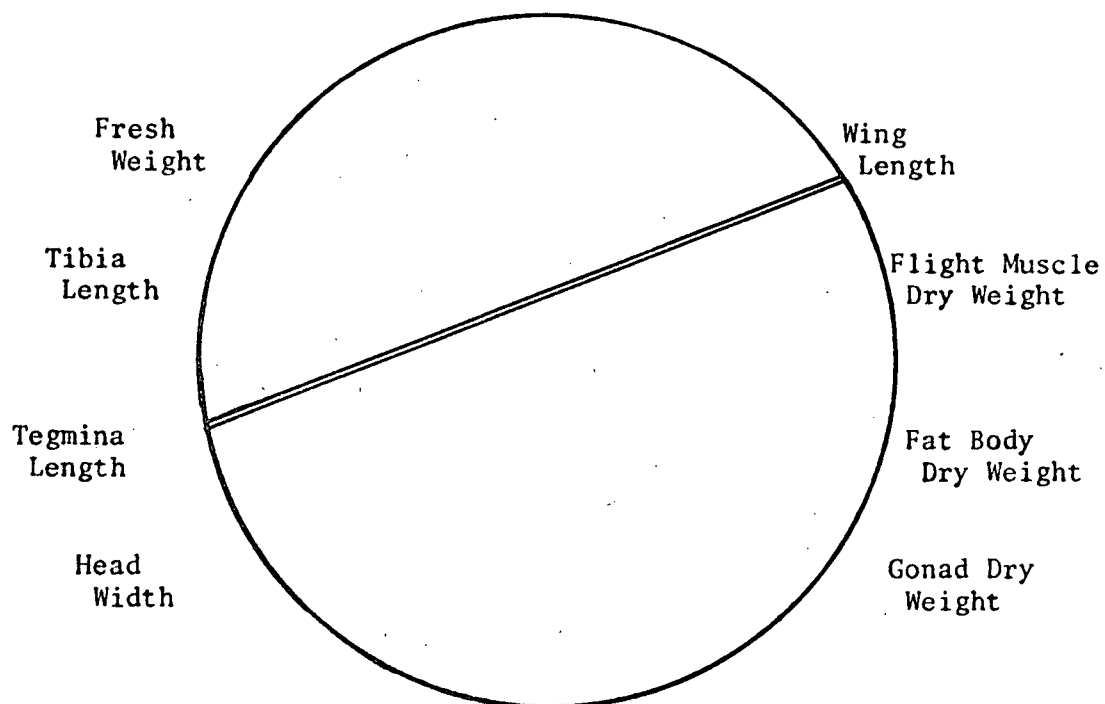
(ii) JHA-treated

FIGURE 13b: The effect of aging and JHA treatment on correlations among body measurements in 14-day-old adult males.

Fresh Weight
Wing Length
Tibia Length
Flight Muscle Dry Weight
Tegmina Length
Fat Body Dry Weight
Head Width
Gonad Dry Weight

(i) Untreated

Fresh Weight
Wing Length
Tibia Length
Flight Muscle Dry Weight
Tegmina Length
Fat Body Dry Weight
Head Width
Gonad Dry Weight
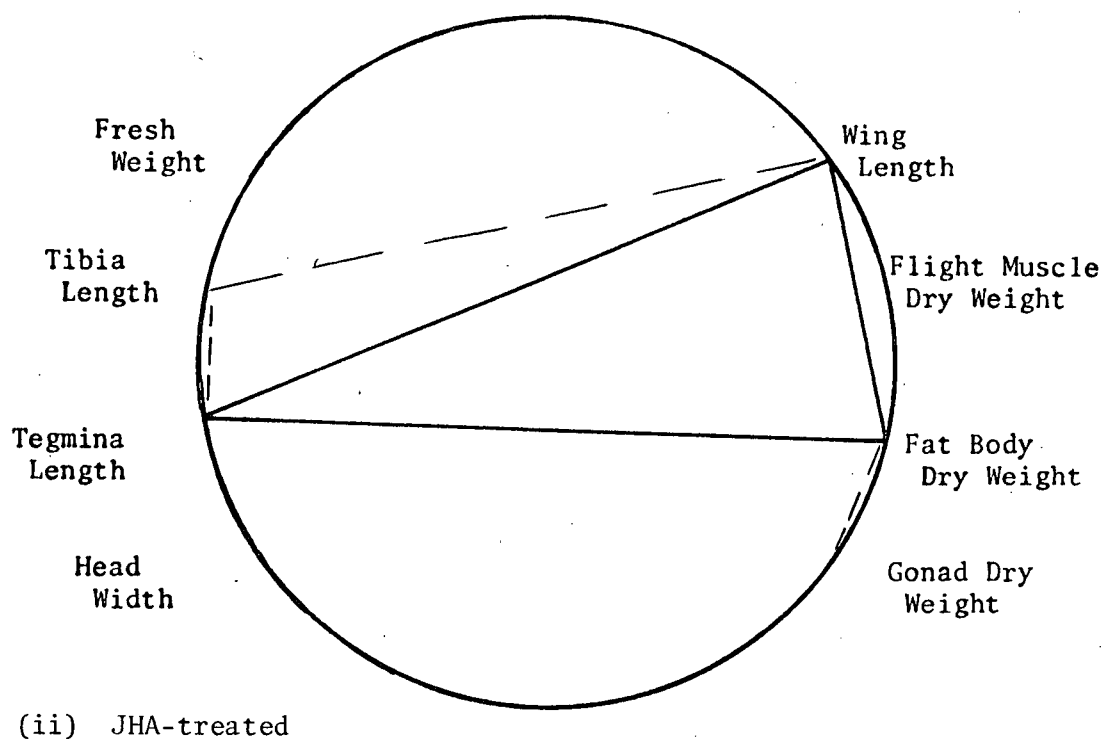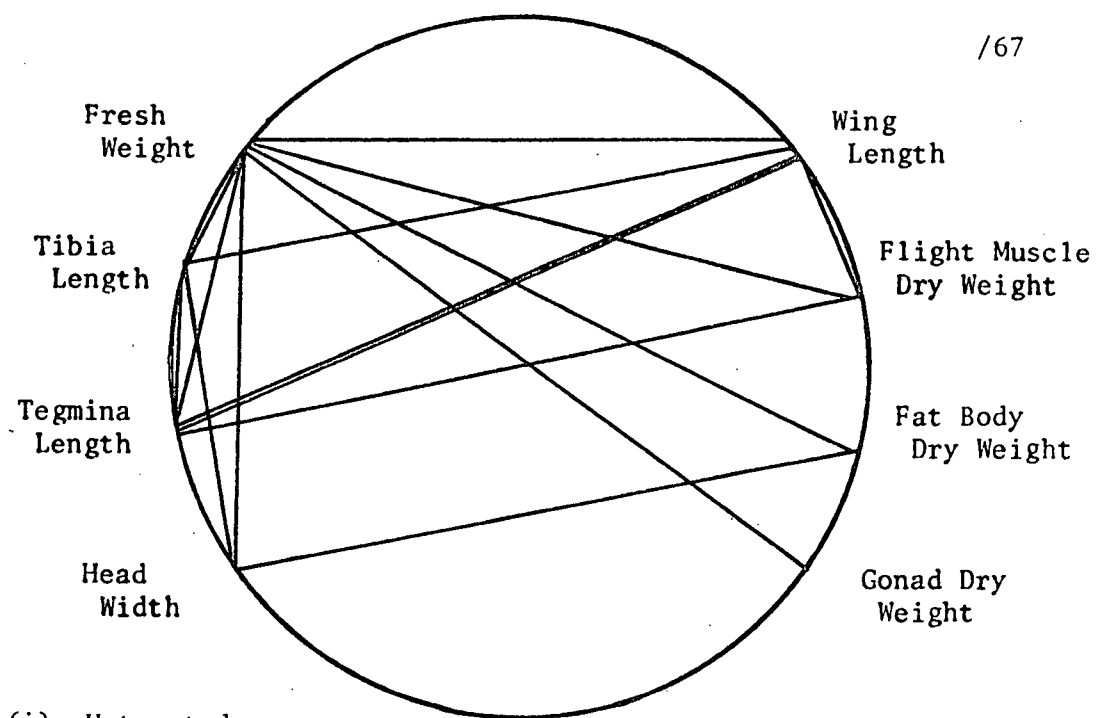
(ii) JHA-treated

FIGURE 13c: The effect of aging and JHA treatment on correlations among body measurements in 5- or 6-day-old adult females.
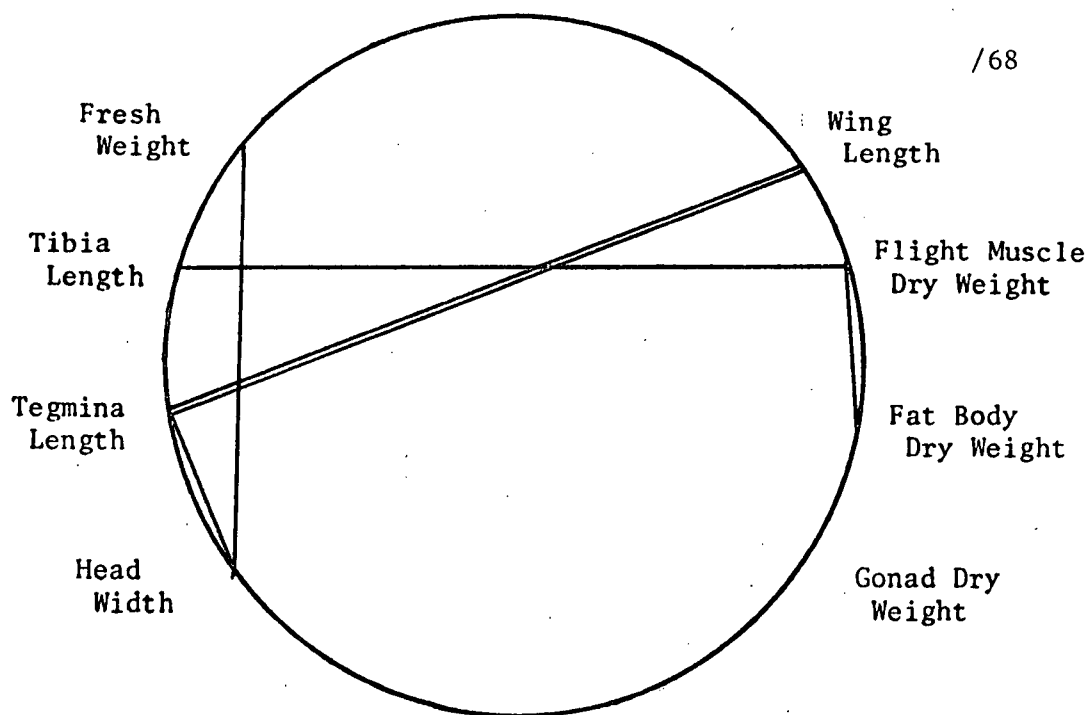
(i) Untreated
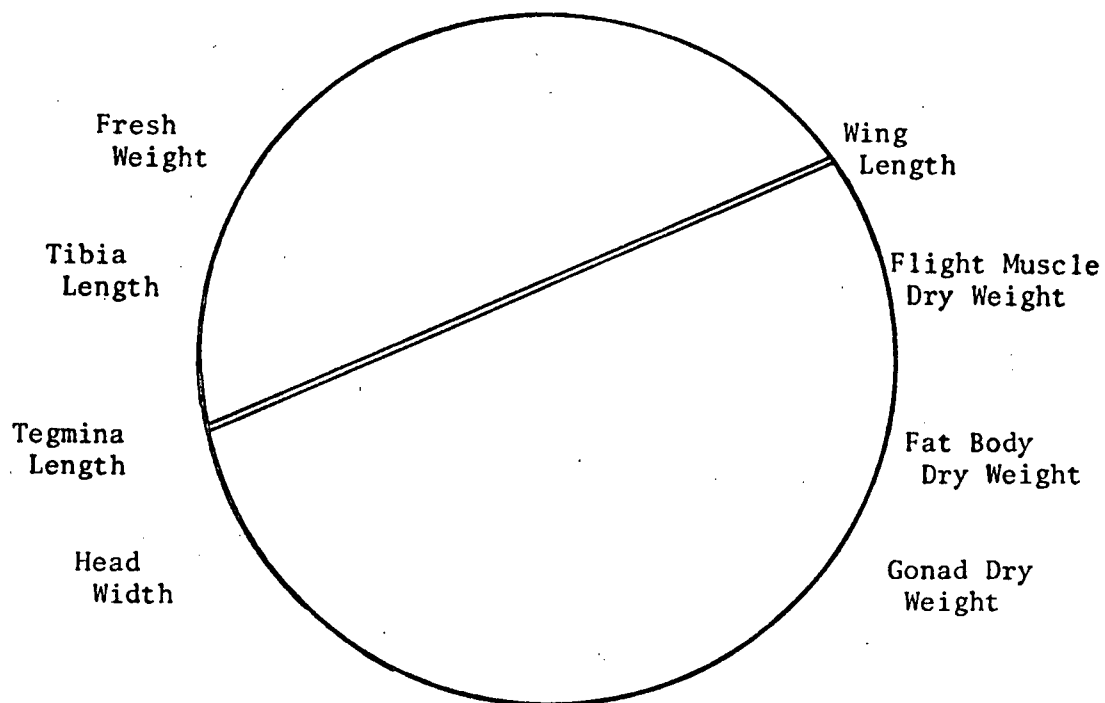
(ii) JHA-treated

FIGURE 13d: The effect of aging and JHA treatment on correlations
among body measurements in 14-day-old adult females.

TABLE XIII: Sensitivity of various stages of *M. sanguinipes* to varying dosages of precocene II

Stage applied	Precocene dose	No. insects (N)	No. dead	No. normal adults	No. abnormal adults	Effects
4th INSTAR						
early	100 µg	8	0	8	0	no effect
early	200 µg	23	7	11	5	precocious metamorphosis
newly emerged	300 µg	8	0	7	1	long wings, no ovarian development
1-day-old	300 µg	7	2	2	2	precocious metamorphosis
4-days-old	300 µg	-	-	-	-	no effect
late	300 µg	-	-	-	-	"
early	400 µg	6	6	0	0	"
misc.	500 µg	6	3	1	2	short wings, juvenile coloring
5th INSTAR						
early	25 µg	5	0	5	0	no effect
late	25 µg	5	0	5	0	"
misc.	50 µg	31	0	31	0	"
early	100 µg	9	0	9	0	"
early	200 µg	27	0	27	0	"
newly emerged	250 µg	6	0	6	0	"
misc.	300 µg	-	-	-	-	"
newly emerged	400 µg	8	1	7	0	"
late	400 µg	3	0	3	0	"
new	500 µg	4	0	3	1	undeveloped ovary
middle	500 µg	7	2	4	1	undeveloped ovary; tegmina longer than wings

(continued)...

TABLE XIII: *(continued)...*

Stage applied	Precocene dose	No. insects (N)	No. dead	No. normal adults	No. abnormal adults	Effects
ADULTS						
young	400 µg	7	0	7	0	no effect
newly emerged	500 µg	5	0	5	0	"
3-4 days old	500 µg	6	0	6	0	"
newly emerged	1000 µg	6	3	0	3	growth retarded slightly
1 day old	1000 µg	5	0	5	0	"
1-4 days old	1000 µg	5	0	5	0	"
3-4 days old	1000 µg	6	0	6	0	"
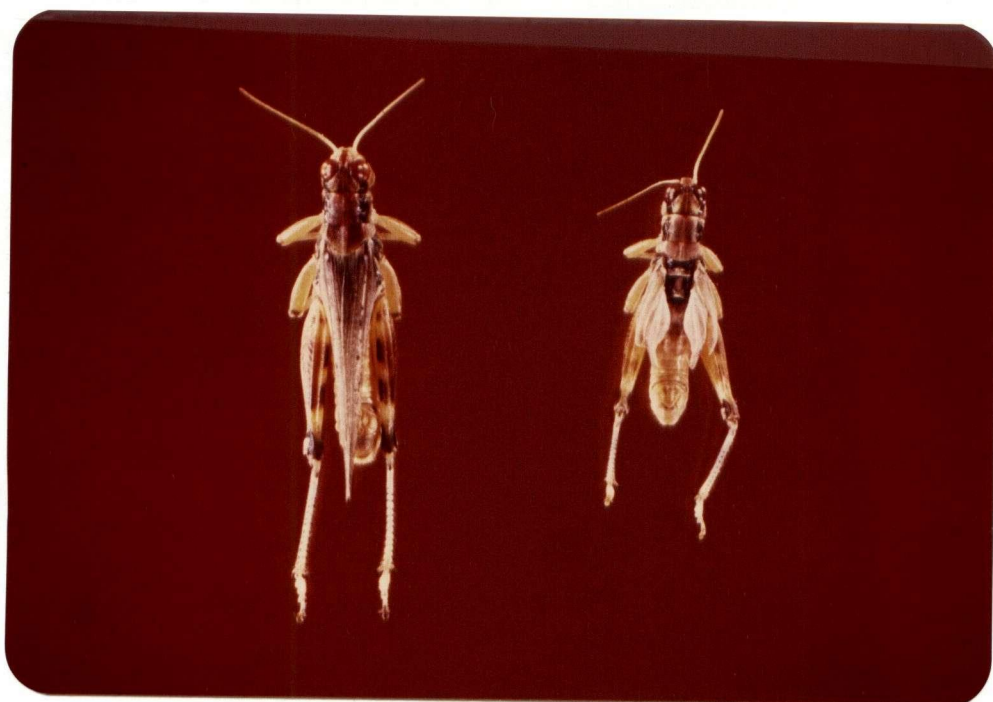
PLATE 6a: Dorsal view of untreated (left) male adult and precocious male adultoid (right) resulting from precocene application (200 μg) to newly emerged fourth instars.
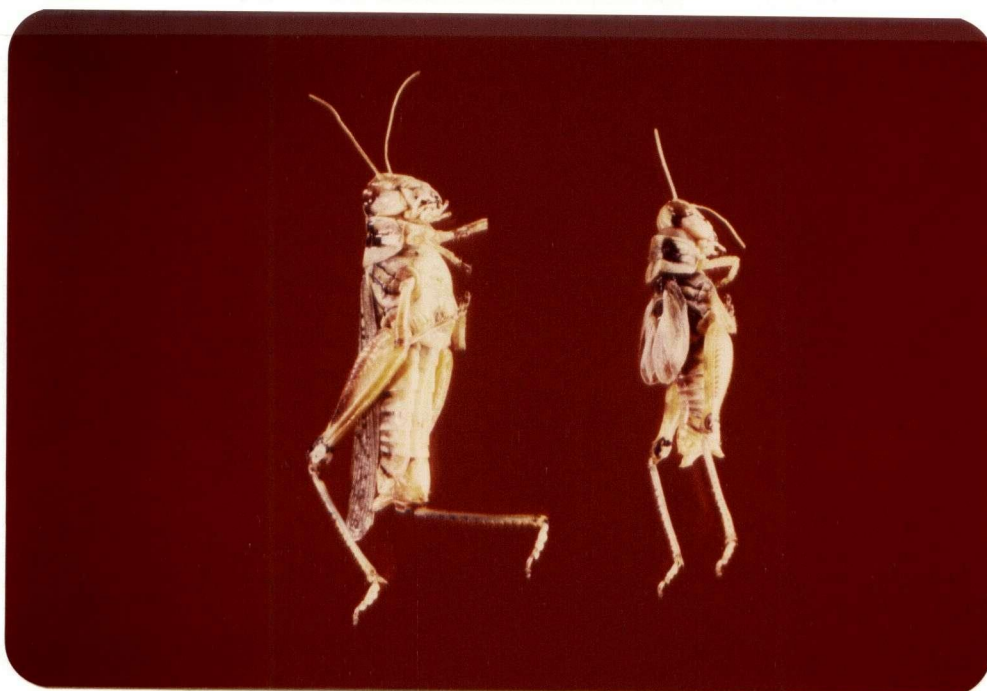
PLATE 6b: Side view of untreated (left) female adult and precocious female adultoid (right) resulting from precocene application (200 μg) to newly emerged fourth instars.

G. *JHA Effects on Precocene-Treated Insects*

(a) *Precocene Effects*

When precocene II (300 μg/insect) was applied to early fourth instar nymphs, approximately 80% of the insects molted precociously into permanent adultoids (pseudo-adults), skipping the fifth instar (Plates 6a and b). These adultoids had fresh weights about half those of normal insects (Table XIV). Gonads, fat body, and flight muscles were also about half normal size.

The precocene-treated adultoids were not only smaller than normal, but their body proportions were also different. Tibia length and head width were about three-quarters that of control insects, while tegmina and wing length were less than half the size of normal appendages.

(b) *JHA Applied to Adultoids*

Table XV shows the effect of a single application of JHA (0.05 μg) to precocious adultoids 6-10 days after the final molt. Significant reductions (P = 0.05) in fat body dry weight were produced in both males and females. In females, ovarian dry weight and total fresh body weight were significantly increased. No significant difference was seen in the size of the fixed sclerites following JHA treatment, except for an apparently significant increase in tibia length.

Figs. 14a and b show the correlations among body measurements in precocene- and precocene-JHA-treated insects.

TABLE XIV: Mean body measurements (± S.D.) of 6- to 10-day-old normal and precocene-treated adults. Precocene (300 µg) was applied to newly emerged fourth instars

Body parameter	Male		Females	
	Untreated	Precocene-treated	Untreated	Precocene-treated
N	6	3	6	2*
Total body fresh weight (mg)	341.7±29.1	160.3±2.7	478.4±64.5	162.3±8.2
Tibia length (mm)	10.2±0.4	8.1±0.1	11.5±0.5	8.0±0.0
Tegmina length (mm)	19.2±1.3	6.5±1.0	20.3±1.0	5.0±0.4
Wing length (mm)	19.2±1.3	6.5±1.0	20.3±1.0	5.0±0.4
Head width (mm)	3.9±0.1	3.1±0.2	4.2±0.2	3.3±0.1
Gonad dry weight (mg)	10.3±1.4	5.1±0.8	37.5±12.8	2.1±0.2
Fat body dry weight (mg)	9.1±2.8	6.4±0.5	17.0±3.9	15.9±2.4
Flight muscle dry weight (mg)	13.2±2.3	6.1±0.4	15.0±3.3	7.0±0.4

* An anomalous female which molted precociously after precocene treatment, and produced mature eggs, was not included in the analysis.

TABLE XV: Effect of 0.05 µg JHA applied after the final molt to precocene-treated adultoids

| Body parameter | Males | | | | | Females | | | | |
	Precocene only Means ± S.D.	Precocene + JHA Means ± S.D.	D.F.	F value	Significance	Precocene only Means ± S.D.	Precocene + JHA Means ± S.D.	D.F.	F value	Significance
N	3	3				2	3			
Total body fresh weight (mg)	160.3±2.7	143.0±12.6	1,5	5.45	.0789 NS	162.3±8.2	190.1±4.1	1,4	27.79	.0133
Tibia length (mm)	8.1±0.0	8.0±0.0	1,5	1.50	.2879 NS	8.0±0.0	8.4±0.1	1,4	18.15	.0237
Tegmina length (mm)	6.5±1.0	6.2±0.8	1,5	0.24	.6476 NS	5.0±0.4	6.9±0.9	1,4	7.14	.0755 NS
Wing length (mm)	6.5±1.0	6.1±0.8	1,5	0.24	.6476 NS	5.0±0.4	6.9±0.9	1,4	7.14	.0755 NS
Head length (mm)	3.1±0.0	3.2±0.0	1,5	0.50	.5185 NS	3.3±0.1	3.3±0.1	1,4	0.36	.5908 NS
Gonad dry weight (mg)	5.1±0.8	4.7±1.0	1,5	0.25	.6453 NS	2.1±0.2	12.9±3.6	1,4	33.41	.0103
Fat body dry weight (mg)	6.4±0.5	3.5±1.8	1,5	6.99	.0574	15.9±2.4	8.6±2.7	1,4	9.45	.0544
Flight muscle dry weight (mg)	6.1±0.4	5.2±1.5	1,5	1.12	.3482 NS	7.0±0.4	5.7±0.9	1,4	3.93	.1416 NS

JHA application increased the correlation amongst body parameters in males but not in females. Comparison of Figs. 6 and 14a and b indicates that there was far less correlation between body measurements in insects which had been treated with precocene than in normal insects.

(c) *JHA Applied After Precocene but Prior to the Next Molt*

Table XVI summarizes the effects of precocene II and subsequent JHA applications applied to fourth instars. Six of the 8 precocene-treated insects molted precociously into adultoids (Plate 7a) while 2 grasshoppers developed into normal adults. JHA applications at different times during the fourth instar stadium resulted in a graded series of morphological effects. When R-20458 was applied to 4-day-old precocene-treated insects, all survivors molted into fifth instar insects (Plate 7b) and eventually into normal-looking adults. However, when the JHA was applied 1 day later in the stadium only 2 of 7 insects eventually molted into normal-looking adults. The remaining insects molted into adult-nymphal intermediates (semi-adultoids) which subsequently died attempting a further molt (Plate 7C). When R-20458 was applied during the sixth day of the stadium, a mixed response resulted (Plate 7d). Two insects died attempting the imaginal molt, whereas 2 molted precociously into adultoids. The remaining 4 insects developed into normal adults.
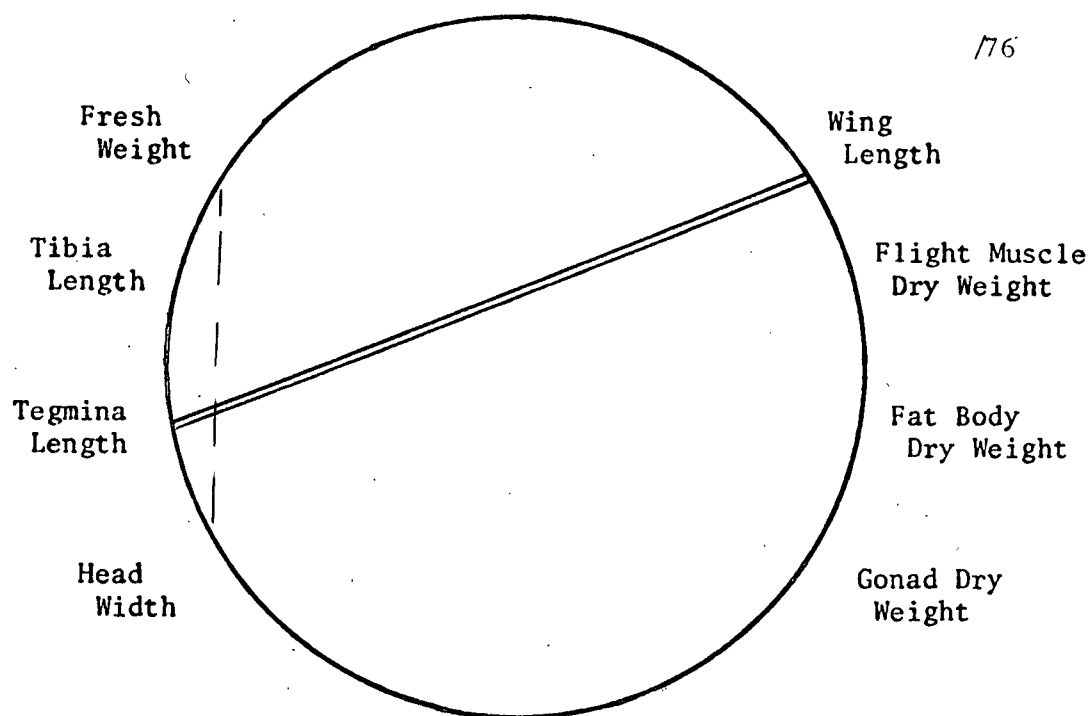
(i) Precocene
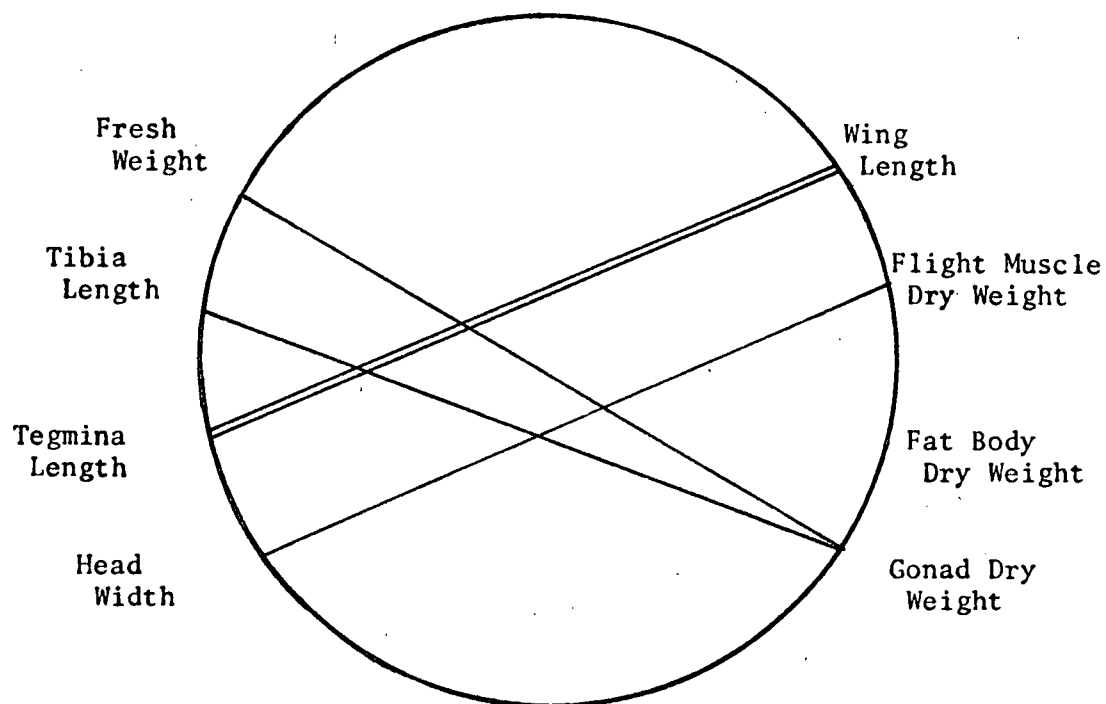
(ii) Precocene + JHA

FIGURE 14a: Correlations among body measurements in male
adultoids treated with precocene II as fourth
instars and with R-20458 after their
precocious molt.
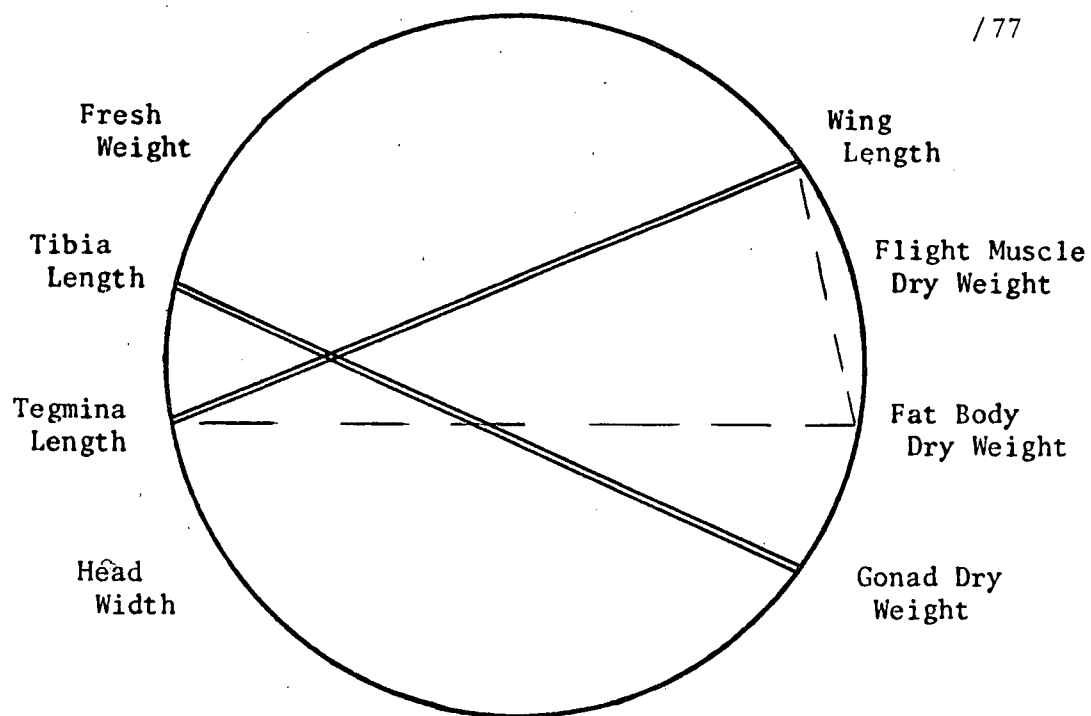
(i) Precocene
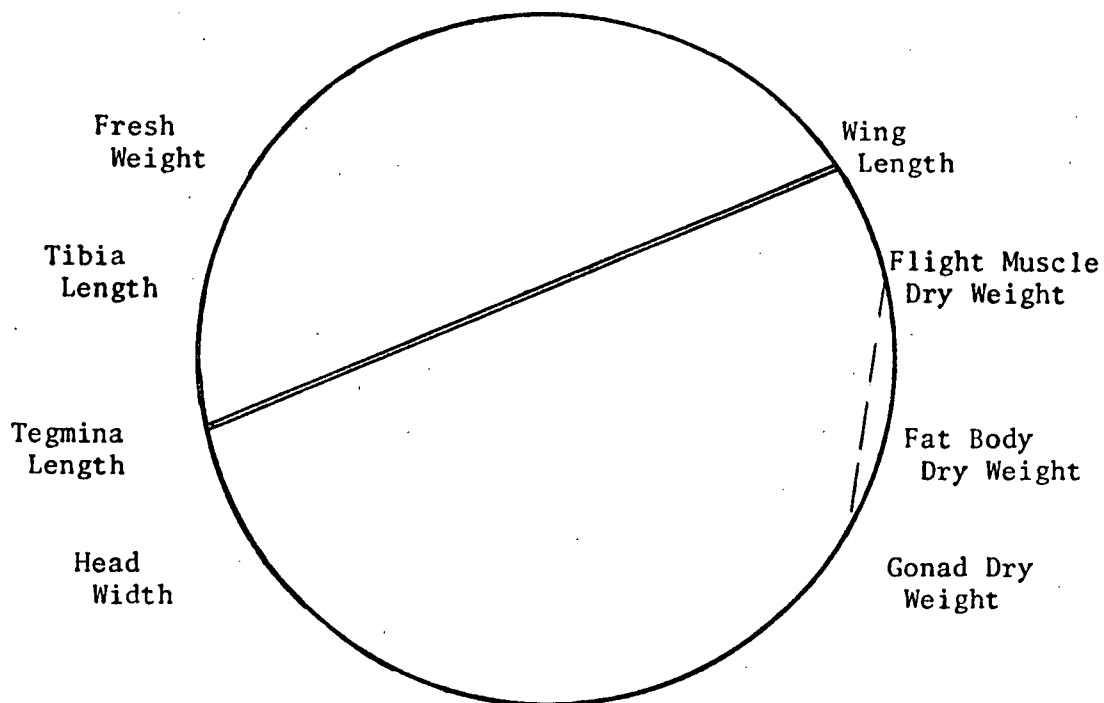
(ii) Precocene + JHA

FIGURE 14b: Correlations among body measurements in female
adultoids treated with precocene II as fourth
instars and with R-20458 after their precocious
molt.

TABLE XVI: Overall effects of 0.05 µg R-20458 applied to precocene-treated
insects at various intervals prior to the next molt

Treatment	# Insects	Mortality	# Adults	# Adultoids	Summary
Precocene only	8	0	♀ 1 ♂ 1	5 1	true adultoids and normal reproducing adults
Precocene + JHA on day 4	8	1	♀ 4 ♂ 3	0 0	all normal-looking adults
Precocene + JHA on day 5	7	5	♀ 1 ♂ 1	0 0	semi-adultoids (died molting), or non-reproducing adults
Precocene + JHA on day 6	8	2 (molting)	♀ 2 ♂ 2	1 1	died molting, or true adultoids, or reproducing adults
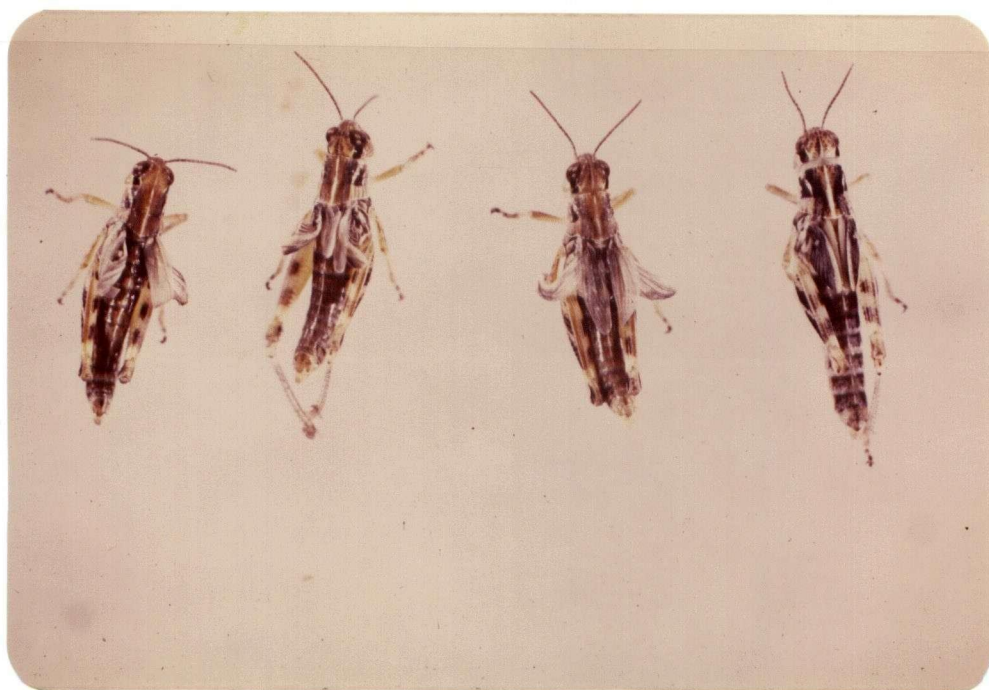
PLATE 7a: Fifth instar nymph (right) and three precocious adultoids (left) resulting from precocene (300 µg) application to 1-day-old fourth instar nymphs.
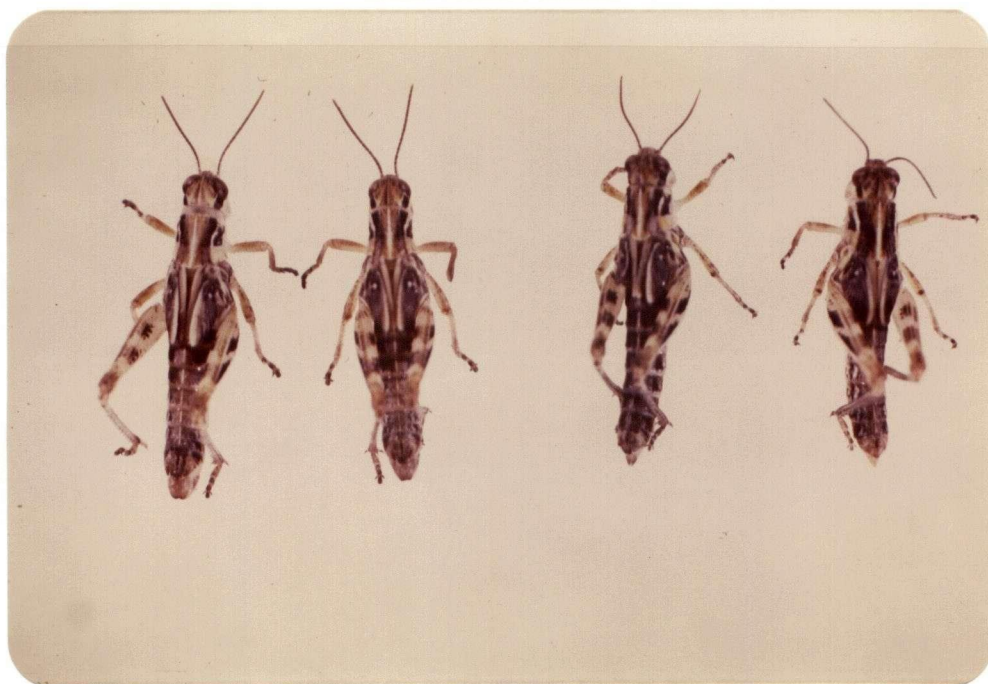
PLATE 7b: Normal-looking nymphs which received a single precocene application (300 µg) as 1-day-old fourth instars followed by 0.05 µg R-20458 on day 4 of the same stadium.
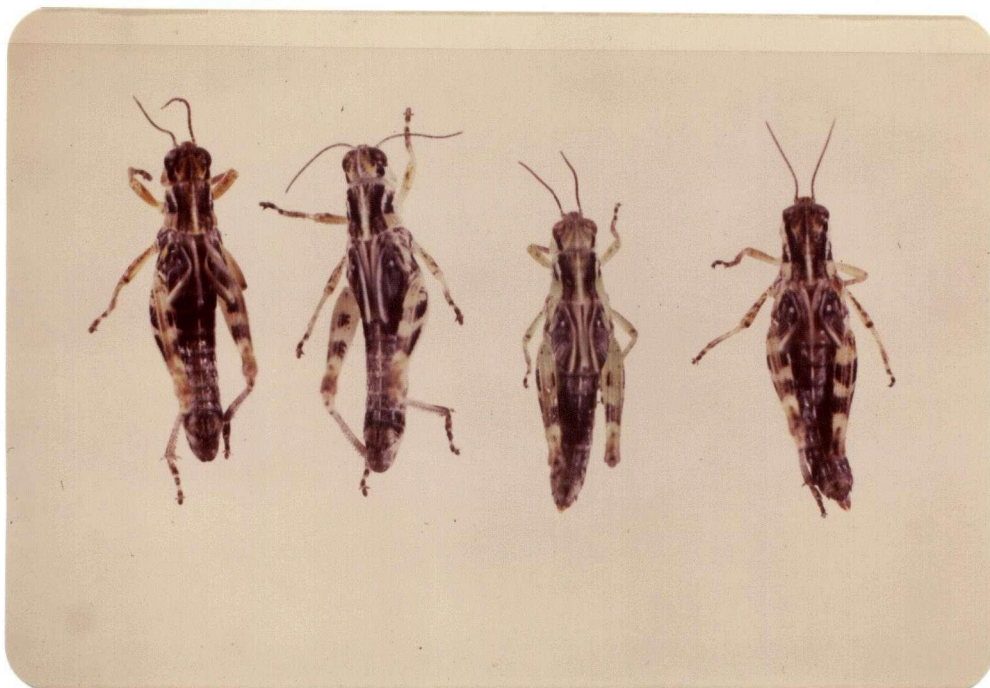
PLATE 7c: Two semi-adultoids (far left and far right) which later died attempting another molt, and two fifth instar nymphs (center) which became non-reproducing adults. The insects were treated as mentioned previously, except that the JHA was applied on day 5 of the fourth stadium.
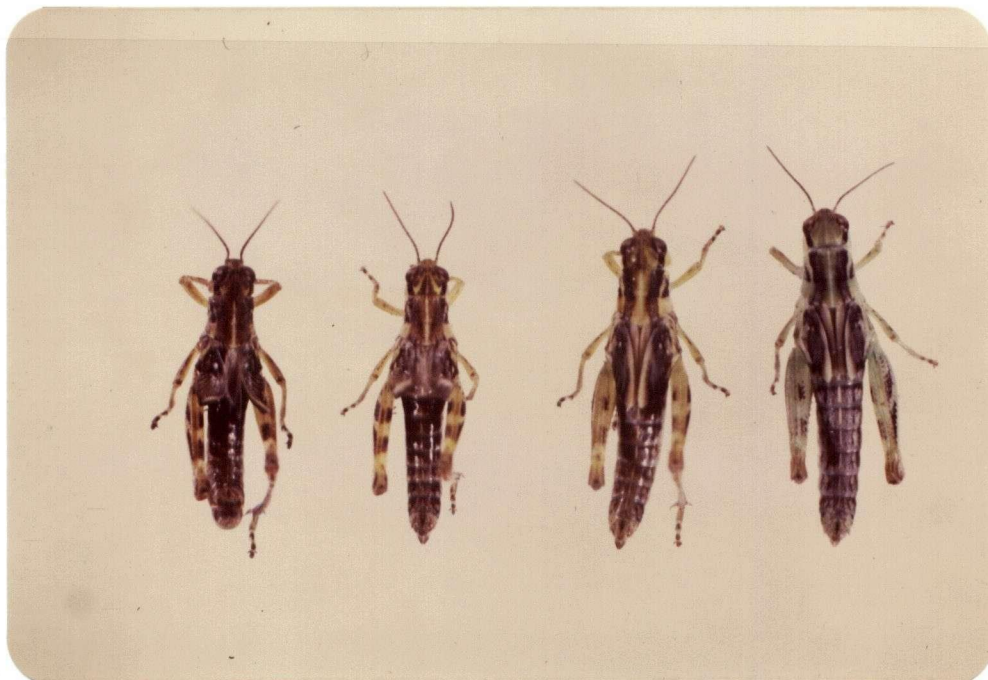
PLATE 7d: Two true adultoids (left) and two fifth instar nymphs which later became reproductive adults. The insects were treated as above, except that the JHA was applied on day 6 of the fourth stadium.

The morphological effects of precocene II and subsequent
JHA applications applied to fourth instars are shown in Table
XVII. When R-20458 was applied to 4-day-old precocene-treated
insects, the surviving adults of both sexes had smaller than
normal tibia length and head width, and shortened tibia and wings.
Mean wing length was also shorter than tegmina length. Total
fresh body weight, flight muscle dry weight, and gonad dry
weight were reduced in both sexes, but particularly in the females.
When the JHA was applied one day later, the insects which molted
into adults exhibited a pronounced reduction in fresh body
weight and gonad dry weight in both sexes. Head width and tegmina and
wing length were slightly reduced, but the tegmina and wings were
of equal length. In the females, the dry weight of the flight
muscles, but not of the fat body, was lower than in normal insects.
In the male, however, both the fat body and flight muscles were
severely reduced. Survivors of R-20458 application to
6-day-old fourth instars previously treated with precocene
produced 2 precocious adultoids similar to those described
previously (Table XIV). However, the flight muscle dry weight of
males was even less than that seen in insects treated with
precocene alone. The 4 insects which became relatively normal
adults nevertheless exhibited shortened wing and tegmina lengths
and slightly reduced flight muscle dry weight, particularly
in the males. Wings were shorter than tegmina.

TABLE XVII: Effects of timed JHA applications to precocene-treated fourth instars. Measurements were taken 4-5 days after adult emergence

	Males					Females				
		Time of JHA application					Time of JHA application			
				Day 6					Day 6	
Body parameter	Precocene only	Day 4	Day 5	precocious	normal	Precocene only	Day 4	Day 5	precocious	normal
N	1	3	1	1	2	5	3	1	1	2
Total body fresh weight (mg)	193.6	270.0±22.7	133.9	143.9	312.0±13.2	178.8±23.8	321.0±22.3	261.2	185.2	399.5±39.2
Tibia length (mm)	8.5	9.6±0.2	10.5	7.8	10.3±0.6	8.1±0.3	10.3±0.9	10.5	9.9	11.1±0.4
Tegmina length (mm)	5.9	16.4±2.3	17.7	6.3	18.4±0.1	6.6±0.6	17.0±1.2	17.2	6.2	19.2±0.7
Wing length (mm)	5.9	15.2±2.7	17.7	6.3	17.4±1.3	6.6±0.6	14.6±0.9	17.2	6.2	18.5±0.7
Head width (mm)	3.4	3.6±0.2	3.6	2.9	3.7±0.1	3.3±0.2	3.8±0.2	3.7	3.2	4.1±0.1
Gonad dry weight (mg)	6.3	7.1±1.1	3.0	4.3	9.3±1.3	2.0±0.5	5.2±5.6	2.2	1.8	40.8±3.0
Fat body dry weight (mg)	9.5	7.6±3.7	0.5	4.0	7.8±0.8	14.3±2.1	15.0±6.4	15.2	14.2	12.4±1.4
Flight muscle dry weight (mg)	7.1	7.5±1.9	2.7	3.7	8.3±1.3	7.2±0.7	8.5±0.9	9.5	5.7	11.7±0.8

DISCUSSION

A. *Normal Development*

As in other acridid species (Pfeiffer, 1945; Hill *et al.*, 1968) newly emerged females of *M. sanguinipes* undergo two distinct phases of development (Gillott and Elliott, 1976). The initial phase of somatic development, which was reported to involve growth of the cuticle, flight muscles and alimentary tract, is completed within 3 days of emergence. After reaching a basic weight, mated females undergo successive periods of reproductive growth that take 2 to 4 days to complete. In the current study, a biphasic growth pattern was demonstrated in both sexes. At all adult stages investigated, the total body weight of males was consistently less than that of females. However, during the somatic growth phase, the overall growth rates, as judged by the regression slopes, were comparable in the two sexes, e.g. b_{male} = 23.8, b_{female} = 26.3. After this period, growth rates differed markedly. In females, the total body weight increased significantly (b = 15.8) until day 8 when oviposition occurred. As reported previously (Gillott and Elliott, 1976; Elliott and Gillott, 1978), this increase is largely due to ovarian growth and protein accumulation by the developing oocytes. In contrast, the total body weight of males did not increase significantly 4 to 9 days after emergence (b = 4.6). The absence of weight increases in the male comparable to those in the female reflects several fundamental differences

in the reproductive physiology of the sexes. Firstly, the dry weight

of the testes-accessory gland complex contributes little to the total

body weight of the male. The complex, which weighed 6 mg on day 3,

reached a maximum of 10 mg on day 5 when the weight stabilized. This

increase is largely attributable to the growth of the accessory glands.

Apart from demonstrating that males become reproductively mature

earlier than females, the stable weight of the testes-accessory gland

complex appears to result from the promiscuous behavior observed in

male *M. sanguinipes* (Friedel and Gillott, 1977). After day 5,

reproductively-active males may copulate several times daily and, on

each occassion, transfer about seven spermatophores into the female

(Pickform and Gillott, 1972). Proteins, which are a major constituent

of the spermatophore, are synthesized in the fat body and sequestered

by the accessory glands prior to mating (Gillott and Friedel, 1976).

Like vitellogenic proteins (Elliott and Gillott, 1979), these

accessory gland proteins are eventually deposited as yolk in the

developing oocytes (Friedel and Gillott, 1977). Therefore, in females,

proteins produced by both sexes accumulate in the ovary throughout

the entire reproductive period until prior to oviposition. In

contrast, multiple copulations by the male result in the daily discharge

of proteinaceous products by the accessory glands. Thus, cumulative

weight changes in the gonads and total body weight observed in

females are not evident in males.

Changes in the dry weight of the fat body in females

concurred with those recorded for female *M. sanguinipes* (Gillott and

Elliott, 1976) and *S. gregaria* (Hill *et al.*, 1968). The dry weight

was small from adult emergence until day 3, when a rapid increase

began. The dry weight peaked at day 5, then decreased until

oviposition occurred. The decrease in the dry weight of the fat body

is accompanied by a rapid increase in ovarian dry weight. Elliott

and Gillott (1976, 1978) have shown that in female *M. sanguinipes*

the CA controls yolk protein synthesis in the fat body and subsequent

protein uptake by the ovary. Therefore, the CA are responsible for

the coordinated development of these two tissues during early

adulthood.

In male *M. sanguinipes*, changes in the dry weight of the fat

body also closely paralleled those of the testes-accessory gland complex

during the first 5 days of adulthood. For the next 4 days, however,

the dry weight of the complex remained fairly constant while that of

the fat body decreased. These results are consistent with the findings

of Friedel and Gillott (1976a,b) who showed in male *M. sanguinipes*

that the fat body aids in spermatophore production by synthesizing

protein for the male accessory glands.

In female *Locusta* (Poels and Bennakkers, 1969) and

Schistocerca (Panar and Nair, 1975), rapid increases in body weight

during early adulthood are associated with rapid flight muscle development.

However, the above studies did not extend into the reproductive period.

In adult female *S. gregaria* such studies by Hill *et al.* (1968) have

shown a rapid, steady increase in flight muscle dry weight until

soon after yolk deposition begins. After this, the weight remained relatively steady. In *M. sanguinipes*, similar changes in the dry weight of the flight muscles in males and females were evident. Flight muscle size increased rapidly during somatic growth, then fluctuated after somatic maturity. Contrary to previous studies on *M. sanguinipes* (Gillott and Elliott, 1976), the present investigation has shown that flight muscle development is not complete by day 3 but that the dry weight increases until day 5 when it declines.

Flight muscle dry weight and protein content were interdependent and followed a nearly-parallel, fluctuating pattern. Similar findings have been reported in *Locusta* (Poels and Beenakkers, 1969; van Marrewijk *et al.*, 1980). The slopes of the regression lines showing the relationship between flight muscle protein content and dry weight in both sexes also indicate that there was more protein per unit weight in small flight muscles than in the large ones. Therefore, some of the increase in flight muscle dry weight around day 5 appears to be due to the accumulation of non-proteinaceous materials.

Protein synthesis in the flight muscles of *Locusta* remains high even after completion of adult development (van Marrewijk *et al.*, 1980). The latter authors have proposed that in *Locusta* the high level of protein synthesis after maturity is due to high flight muscle metabolism and turnover of tissue materials.

In both sexes of *M. sanguinipes*, peaks in the dry weight of the flight muscle roughly corresponded to those of the fat body.

The dry weight of these tissues in both sexes decreased as yolk

deposition proceeded in the females. A similar trend is found in

several other insects which exhibit high teneral flight activity,

followed by flight muscle autolysis (Chapman, 1969). At this time,

fat body size increases and adult reproductive development begins. *"In*

general it is believed that the degenerating muscles provide essential

reserves for egg development..." (Chapman, 1969). However, *M. sanguinipes'*

flight muscles do not completely degenerate, they merely decrease in

size prior to the first oviposition. It would be interesting to see

if materials from the flight muscles are incorporated into the fat

body to be used in spermatophore or ovarian development. Analysis

of protein in the flight muscles using methods similar to those of

Gillott and Friedel (1976), would provide a firmer indication of

the potential role of the flight muscles in reproduction.

In the present studies, the significant correlation among

the dry weights of the gonads, fat body, and flight muscle in normal

grasshoppers of each sex emphasizes the coordinated growth patterns of

these organs within individual insects. In addition, the similarity

in growth patterns of the internal organs such as fat body and flight

muscles of males and females may indicate some type of synchronization

between the two sexes. The maturity of male locusts and grasshoppers

is known to influence the developmental rate of females (Riegert,1965),

possibly by means of pheromones (Doane, 1973). Interestingly, males

may also contribute to the developing oocytes by transferring protein

to the females via spermatophores (Friedel and Gillott, 1977). If the
fat body and flight muscles of both males and females were contributing
proteins to the oocytes, similar patterns in the development of these
internal organs in the two sexes would be expected. There is even
recent evidence that JH can be passed from the male to the female
during mating in some insects (*H. cecropia*; Shirk *et al.*, 1980). If a
comparable situation exists in *M. sanguinipes*, it would account for the
synchrony of development between the two sexes. Although the influence
of the CA on flight muscle development has not been established in
M. sanguinipes, JH is known to affect the development of the fat body
and gonads. Therefore, it may be that coordinated changes in JH
levels within a grasshopper or locust population would result in
synchronzied development.

B. *Role of JH in Development*

The CA are known to play a critical role in insect development
(Doane, 1973). The classic experiments which Wigglesworth performed
on *Rhodnius prolixus* during the 1930's demonstrated that when the CA
of young nymphs were surgically inactivated (decapitation, extirpation),
the insects molted prematurely into adults. In contrast, when the
CA in final instar nymphs were artificially activated (CA implantation),
the nymphs underwent an extra nymphal molt before metamorphosis into
the adult stage. These studies prompted Wigglesworth to hypothesize
that elevated JH titres in the haemolymph of younger nymphal stages

suppresses adult differentiation and favours the retention of juvenile characteristics. Conversely, a precipitous decline in JH haemolymph titres during the final instar results in metamorphosis and the expression of adult characteristics. Confirmation of this hypothesis in *Locusta* males and females has been provided by more recent studies which have indirectly (*Galleria* bioassay) or directly (radio-immuno assay, electron-capture gas chromatography) measured JH haemolymph levels during nymphal development (Johnson and Hill, 1973a,b, 1975; Baehr *et al.*, 1979; Huibregtse-Minderhoud *et al.*, 1980). Collectively, these studies have shown that high JH levels are present in the haemolymph throughout most of the fourth (penultimate) stadium whereas JH is absent throughout most of the final stadium. However, Baehr *et al.* (1979) reported a temporary surge in JH levels at the beginning of the fifth instar.

In acridid species, JHA and precocene have been employed as chemical probes to artificially manipulate JH levels during nymphal and adult development. In *M. sanguinipes*, the effects of these compounds on development depend upon the stage to which they are applied. As reported in other species (Kelly and Fuchs, 1978; Kruse Pedersen, 1978; Unnithan and Nair, 1979), topical application of 200-300 µg precocene II to recently molted fourth instar nymphs of *M. sanguinipes* resulted in a high proportion of the insects molting precociously into diminutive adults. Precocene II tends to have a variable success rate, and 50% effectiveness is common (Muller *et al.*,

1979; Masner *et al.*, 1980; Unnithan *et al.*, 1980). The precocene apparently inactivates the CA (Unnithan and Nair, 1979), and without sufficient endogenous JH, the insects are unable to maintain juvenile characteristics for a fifth stadium. However, when even higher precocene doses were applied to fifth instar *M. sanguinipes*, molting and metamorphosis were not disrupted. Comparable findings in *Oncopeltus* led Unnithan and Nair (1979) to theorize that precocene only affects insects during those developmental stages in which the CA are active. If this theory is tenable in *M. sanguinipes*, the results of the present study substantiate the premise that JH is absent during part or most of the fifth instar stadium.

Two of 6 insects treated with 500 µg precocene as fourth instars and 1 of 7 insects receiving the same dose as mid-fifth instars retained juvenile characteristics as adults. The effects resembled those of R-20458 and included shortened wings, uneven tegmina and wings, and juvenile coloration. Characteristics of JH excess preceeding CA degeneration have also been observed when comparable stages in *Locusta* were treated with precocene (Fridman-Cohen and Pener, 1980; Miall and Mordue, 1980). These results led Miall and Mordue (1980) to suggest that *"the effect is probably the result of synthesis or release of JH during the breakdown of the glands"*.

C. *Reversing Precocious Metamorphosis with JHA*

Precocious metamorphosis induced by precocene in *Locusta* (Kruse Pedersen, 1978) and *Oncopeltus* (Masner *et al.*, 1979) has been reversed

by applying JHA during the same stadium. However, in both species,

observations were only carried out into the fifth instar so that the

effect on adult morphology was not determined. In the present experiments,

a gradation of effects was observed when 0.05 µg R-20458 was applied

to fourth instars 4 to 6 days after they were treated with precocene II.

When JHA was applied 4 days after the precocene treatment, the insects

molted into fifth instars and eventually into normal-looking adults.

Apparently the dosage of R-20458 applied at this time was high enough

to maintain the juvenile characteristics into the fifth and final

nymphal stadium but also low enough so as not to interfere with

metamorphosis. The same JHA treatment applied one day later resulted

in a high number of semi-precocious adults which later died attempting

another molt. R-20458 applied at this time seemed to be too late

to program a fifth nymphal stadium. Instead, the epidermal cells

received conflicting instructions. Initially, the absence of JH

caused by precocene appeared to result in the epidermal cells being

committed to produce an adult cuticle but later being programmed

by the JHA to produce nymphal cutical and undergo an additional molt.

Results of the present study agree with those of Kruse Pedersen (1978),

who noted that timing of JHA application in such experiments was

critical in *Locusta*, and that the optimum time for JHA-reversal of

precocious metamorphosis was between the third and fourth days of

the fourth stadium. In addition, the current results indicate that

during normal development, JH levels must be low on the third and

fourth days of the fifth instar stadium. However, when JHA was
applied 6 days after the precocene treatment, a mixed effect was observed.
The two insects which died attempting a final molt were probably
still young enough to be affected as in the previous study. However,
two other insects became precocious adultoids, so that the JHA
was applied too late to counteract the effects of precocene. It is
difficult to explain why the other four insects molted to normal-looking
adults, unless JH application immediately before the molt can also
initiate nymphal commitment, which seems unlikely.

D. _JHA Studies on Molting and Metamorphosis_

JHA-induced supernumerary molting has been observed in
Zonocerus (McCaffery and Page, 1978) and in _Locusta_ (Vogel _et al._,
1978). CA implants produced similar effects in _Locusta_ and _Schistocerca_
(Nemec, 1970; Van den Hondel-Franken _et al._, 1980). None of these
papers dealt with the precise JH application time necessary to produce
an extra nymphal molt. Theoretically, if the CA are inactive
throughout part of the fifth instar stadium, then timing of JHA
applications should be important in determining the resulting effects.
This is particularly true since JH-sensitivity of different tissues
in insects varies with development time (Luscher _et al._, 1971; Willis,
1974). In _M. sanguinipes_, supernumerary molting, a symptom of JH-excess,
occurred in approximately one quarter of the insects treated with
0.0375 to 0.375 µg R-20458 in the middle of the fifth stadium. However,

no supernumerary molts developed when R-20458 was applied to early or late fifth instars. Apparently JHA applications during the mid-fifth instar stadium are capable of preventing the commitment of epidermal cells and other tissues to adult development. This again suggests that low JH titres during the middle of the final stadium are necessary to permit an imaginal molt. However, the present results also indicate that high JH levels during the end of the final stadium are inconsequential in terms of molting and metamorphosis.

Locust size is influenced by JH levels. High endogenous JH titre such as those presumably present in *solitaria* locusts, favor increased sexual dimorphism with the development of large females and small males (Kennedy, 1961; Poels and Beenakkers, 1969; Beenakkers, 1973; Beenakkers and Van den Broek, 1974). This differential effect of JH on the two sexes was also observed in the present experiments. JHA application to fifth instar female *M. sanguinipes* produced no significant overall change in fresh body weight of the adult, whereas in males, the fresh body weight of the adults was significantly reduced. Contrary to the conclusions of Senhal (1971), the current findings clearly indicate that JH can serve as a general growth hormone during nymphal development.

Correlations among adult body measurements were greatly reduced after precocene or JHA treatment of nymphs. Therefore, not only the size of the insects, but also the body proportions changed and became less related. Although precocious adults generally looked

normal (except for the tiny wings), there were small changes in relative body measurements. However, Bowers *et al.* (1976) writes that in *Oncopeltus* "*the morphology and coloration of the precocious adults is identical with that of normal adults*". Later additions of JHA to precocious adult males increased the correlations among body measurements, as flight muscle and gonad dry weights were more closely proportional to the size of some of the fixed sclerites. However, female precocious adults, treated with JHA, showed no improvement in body measurement correlations. These results indicate that in males, JH stimulates development of internal organs until they reach a basic relatively stable level in proportion to the other body parameters. However, in females, JH stimulates ovarian dry weight independently from that of most other body parts.

The metamorphic and coloration changes that occur with phase change in many species have been attributed to JH (Table 1). Characteristics of the relatively sedentary *solitarious* phase have been produced by CA implants or JHA applications (Joly, 1960, cited in Uvarov, 1966; Staal, 1961; Doane, 1973). CA-implanted or JHA-injected grasshoppers (Rowell, 1967) and locusts (Kruse Pedersen, 1978) usually turned green after the next molt although green haemolymph could be observed in some cases within the same instar (Rowell, 1967). Results of the present experiments agree with these findings. Reasons for this time lag are probably complex, but may be due in part to the natural cycle of endogenous JH levels within the insects. In

the present experiments, JHA treatment of final nymphal instar males and females produced a variety of coloration and wing length effects, depending upon when the JHA was added. Males and females treated with 0.05 µg R-20458 just after the middle of the fifth instar (days 4-5) became green adults, while those treated on day 6 were only faintly green or normal-colored. Insects treated with JHA on day 3 became normal-colored adults, while those treated on the first 2 days of the instar retained juvenile black and beige markings as adults.

Wing length was also altered when JHA applications were made at various times during the fifth nymphal stadium. Effects on insects treated at the beginning of the fifth instar (newly emerged to day 3) ranged from little effect to pronounced metathetaly. Grasshoppers treated on days 3-4 had a normal appearance, where those treated towards the end of the fifth instar (days 4-5) possessed shortened wings. By day 6 JHA application was too late to produce much effect, and the insects became nearly normal adults. Apparently JH is involved in determining wing length in *M. sanguinipes* at the beginning of the fifth stadium and around days 4-5, but not during the remainder of the stadium. Interestingly, these two periods correspond with the JH peaks in fifth stadium *Locusta* (Baehr *et al.*, 1979). If such peaks are also present in *M. sanguinipes*, the addition of JHA during periods of high endogenous JH titre might be implicated in the production of short wings. If JH must be low in mid-fifth instars to allow the imaginal molt, and since no effect on color or wing length is produced

at this time, it appears that both endogenous JH and JHA applications
are needed to affect these two parameters.

In *M. sanguinipes*, JHA applications can bring about the locust
solitarious phase characteristics of shortened wings and juvenile
coloration. Therefore, *M. sanguinipes* must have the genetic potential
to undergo phase changes in response to increased JH titres at
particular nymphal stages. Results of the present JHA experiments
are supported by similar effects of timed JHA applications to final
nymphal instar *Locusta* and *Schistocerca* (Nemec, 1970; Joly and Meyer,
1970).

Nemec (1970) categorized the JHA effects on locust species
into three groups, including (1) an action on metamorphosis,
(2) changes in phase coloration, and (3) no effect. He concluded
that JHA induces multiple effects and that *"merely the time of
application determined whether a block of metamorphosis or the phase
change will occur"* (Nemec, 1970). He also stated that the JHA must
break down within the insects' bodies in order for the two different
effects to occur. The latter hypothesis fails to consider the
possibility that JHA, even when directly applied at a later stage in
the instar, may be too late to produce metamorphic effects in these
insects. It may even be that JH must be maintained at a certain level
for a particular period of time for various observable effects to occur
(Van den Hondel-Franken *et al.*, 1980). Unfortunately, it is
impossible to discern from the present experiments and those of
Nemec (1970) the extent to which JHA persistence is influencing
the results. Differences between the results of Nemec and the present
experiments may be due to species characteristics or to the JHA

applied. In the present investigation, there was considerable variation of effects between trials. Since locust development is extremely sensitive to environmental conditions, difficulty in obtaining repeatable results is experienced (Beenakkers and Van den Brock, 1976).

Rowell (1967) succeeded in producing green coloration in four grasshopper species, some of which never produced green adults in the wild. However, the CA implants rarely produced metathetalic individuals. The relative absence of this type of effect may be due to the lack of consideration accorded the timing of the implants within the instar in Rowell's experiments. This oversight places in doubt the conclusions of Staal (1961), supported by Rowell (1967), *"that corpus allatum hormone levels in Locusta had profound effects on green/brown polymorphism, but relatively slight effects on morphometrics...it seems clear that there must be in the locusts an inhibitory mechanism responsible for green coloration and that responsible for the gregarious phase, but it cannot be therefore assumed that these effects are opposite extremes of a continuous series"*. In fact, the present experiments and those of Joly and Meyer (1970) and Nemec (1970) showed that JH causes both coloration and morphological effects depending upon application time. It may be that timing of endogenous JH peaks relative to tissue development in each individual insect, is responsible for the wide variety of effects observed in *M. sanguinipes* and other species (Riegert, 1965; Beenakkers and Van den Brock, 1974).

E. JH Effects on the Development of Gonads, Fat Body, and Flight Muscles

High JH levels caused by CA implantations into young fifth
instar *Locusta* females have been implicated as a cause of poor flight
muscle development (Poels and Bennakkers, 1969; Van den Hondel-Franken
et al., 1980). In the present experiments, JHA applications to fifth
instar *M. sanguinipes* of both sexes produced a significant (P = 0.01)
reduction in the flight muscle dry weight. However, no significant
difference was produced in flight muscle dry weight by varying JHA
application time within the instar. Apparently the flight muscles of
M. sanguinipes are JHA-sensitive throughout the fifth instar stadium,
even though wing length is only affected at certain times within the
stadium. Results of the present experiments do not support the
theory of Chudakova *et al.* (1976) that *"the flight apparatus develops
as an integrated functioning system rather than as a result of im-
aginisation of individual components"*. After JH treatment, the
correlation between various body measurements is usually reduced,
indicating that each tissue responds in a unique way to the hormone.

Adding JHA to precocious adultoids did not significantly
affect flight muscle dry weight. Apparently JHA added after early
adulthood is too late to affect flight muscle growth. This indicates
that previous commitment of the tissues may be important in the case
of flight muscles, although not of the fat body as mentioned below.
It seems that after early adulthood, JHA cannot alter flight muscle
synthesis even though it is capable of stimulating fat body and

ovarian development at that time. The presence of some type of programming within insect muscles which determines the timing of their response to hormones has also been suggested by Poels and Beenakkers (1969), Unnithan and Nair (1977), and Van den Hondel-Franken *et al.* (1980).

Bioassays of haemolymph JH levels in gregarious adult male *Locusta* and *Schistocerca* (Johnson and Hill, 1973b) and adult female *Locusta* (Johnson and Hill, 1975) indicated that in both sexes the hormone was present in relatively small amounts immediately after imaginal ecdysis. However, JH then became almost undetectable until just before the beginning of sexual maturation which began in both sexes around day 10. Following sexual maturity, JH levels in males remained uniformly high whereas in females the hormone level peaked near oocyte maturation and dropped to a low titre at oviposition. Subsequent chromatographic studies confirmed the presence of JH in 18-day-old mature adult female *Locusta*. In an *in vitro* radiochemical assay on excised CA from adult female *Schistocerca*, Tobe and Pratt (1975) showed that JH peaks near the onset of vitellogenesis of each successive egg batch.

The studies of Elliott and Gillott (1976, 1978, 1979) strongly indicate that in adult female *M. sanguinipes* the CA stimulate the synthesis of yolk proteins and their uptake by the ovary. JH is also known to enhance reproductive functions in male adult grasshoppers of this species (Gillott and Friedel, 1976). It is possible,

however, that high JH levels in the late larval stages also affect

gonad maturation, since in *solitaria* locusts the rate of reproductive

development is usually different from that of *gregaria* (c.f.

Kennedy, 1961). When JHA was applied to fifth instar *M. sanguinipes*

of both sexes, gonad dry weight increased or decreased. Attempts

to initiate reproducible JHA-effects on gonad dry weight did not produce

clear consistent trends, even though the insects within each jar

usually exhibited similar development. Overall, gonad dry weight

of males and females was significantly reduced in the first, but not

in the second trial. These results indicate that precise timing of

JHA applications to fifth instars relative to each insect's stage of

development results in a wide range of effects on the gonads of both

sexes.

With the intention of lowering JH titres by inactivating

the CA, 300 µg precocene II was applied to 1-day-old fourth instar

nymphs. In the supposed absence of JH, most of the precocious adult

insects were apparently sterile, possessing greatly reduced reproductive

organs. The single exception was an anomalous precocious female

which contained eggs. Sterility is a common result of precocene

treatment (Bowers *et al.*, 1976) in both males and females. As

reported in *Oncopeltus* (Bowers *et al.*, 1976; Bowers and Martinez-Pardo,

1977; Unnithan and Nair, 1979), JHA reversed the sterility of female

M. sanguinipes. However, after JHA was applied to precocious adult

male *M. sanguinipes*, no significant change in gonad dry weight was

observed. This indicates that in males of this species, JH must be present during nymphal or early adult development for normal gonad size to be reached. Gonad size in males does not increase if JHA is added after early adulthood.

The reduction in general fat body dry weight after JHA application, seen in the present experiments, was also recorded in *Locusta* after CA implantation into final stadium nymphs (Poels and Beenakkers, 1969), and in allatectomized females *M. sanguinipes* adults after JHA treatment (Gillott and Elliott, 1976; Elliott and Gillott, 1978). In *Locusta*, the extra JH produced no observable effect until after the imaginal molt. The present experiments show, however, that in *M. sanguinipes* the fat body is also susceptible to JH during the fifth instar. Although JHA treatment of fifth instar males and females generally had no significant influence on fat body dry weight, the effects of varying application time within the fifth instar were highly significant. In determining the amount of subsequent fat body reduction, precise timing of JHA application is critical but no clear-cut period of JHA-sensitivity of the fat body was observed. As in the previous JHA trials on normal fifth instars, fat body size decreased significantly when precocious adults of both sexes were treated with JHA. Therefore, the development of the fat body of *M. sanguinipes* is not entirely pre-programmed because the tissue is sensitive to JHA after adulthood is reached.

As adult *M. sanguinipes* males and females age, the effects
of the JHA treatment during the fifth stadium appear to decrease.
Correlation among body measurements also decreased within each group
of insects as they aged, regardless of treatment. Synchronization
of development appears to be most important during early adulthood.
Such a system would favor locust survival since large numbers of
insects must develop simultaneously for swarming to take place.
After reproductive development occurs, swarms disperse, and coordinated
maturation rates are no longer necessary. Apparently, a similar loss of
developmental synchrony also takes place in grasshoppers.

F. *Grasshopper Control*

Depending upon the timing of the application, both precocene
II and R-20458 have been shown to drastically alter the development
of various tissues in *M. sanguinipes*. The present studies have
substantiated previous reports that JH regulates fat body metabolism
and the development of the gonads. In addition, JH has been shown
to regulate somatic growth, particularly that of the wing and flight
muscles. Depending upon when JHA and precocene were applied, the
development of the tissues was so drastically altered that the
compounds were lethal to the grasshoppers. Therefore, theoretically,
the compounds are potentially insecticidal and, in addition, act
as chemosterilants or inhibit wing development. However, the wide
variety of effects produced by changes in critical timing of both
precocene and JHA appliations makes these compounds unlikely as agents

of grasshopper control, at least at the present time. Grasshopper
emergence is asynchronous and the insects' habits are solitary.
Timing of sprays relative to the insects' development would be
critical. In addition, grasshoppers have time to cause significant
economic damage before they reach the final nymphal stadium in which
they are most susceptible to the lethal effects of JHA.

LITERATURE CITED

Albrecht, F. O., Michel, R. and Casanova, D. (1978) The temperature and photoperiodic control of flight activity in crowded desert locusts, *Schistocerca gregaria* (Forsk.). II Changing photoperiods. General discussion on the acquired flight ability of locusts. Acrida 7: 289-298.

Anonymous (1970) Proceedings of the International Study Conference on the Current and Future Problems of Acridology. (Ed. by C. F. Hemming and T. H. C. Taylor) Center for Overseas Pest Research, London, 1972. Eyre and Spottiswoode Ltd., London.

Baehr, J-C, Porcheron, P., Papillon, M. and Dray, F. (1979) Haemolymph levels of juvenile hormone, ecdysteriods and protein during the last two larval instars of *Locusta migrataria*. J. Insect Physiol. 25: 415-421.

Baker, G. T. (1976) Insect flight muscle: maturation and senescence. Gerontology 22: 234-261.

Barker, J. F. (1979) Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*. Experientia 35: 552-554.

Baron, S. (1972) The Desert Locust. Scribner's, New York. pp. 1-42, 197-223.

Beenakkers, A. M. Th. (1973) The influence of corpora allata on
flight muscle development in locusts. J. Endocrinol.
57: LII.

Beenakkers, A. M. Th. and Van den Broek, A. Th. M. (1972) Influence
of juvenile hormone on growth and digestion in fifth instar
larvae and adults of *Locusta migratoria*. J. Insect Physiol.
20: 1131-1142.

Benson, J. and Oberlander, H. (1974) Protein synthesis in wing discs
of *Galleria mellonella*: effects of ecdysone and fat body
in vitro. Insect Biochem. 4: 423-428.

Bethune, C. J. (1874) Reports on some of the noxious and beneficial
insects of the Province of Ontario. Ann. Rep. Ent. Soc.
Ont. 5: 29-42.

Blight, M. M. and Wenham, M. J. (1976a) Juvenile hormone activity
in larvae and adult females of the locust, *Schistocerca
gregaria*. J. Insect Physiol. 22: 141-145.

Blight, M. M. and Wenham, M. J. (1976b) Identification of JHIII in
haemolymph from adults and larvae of *Schistocerca gregaria*.
Insect Biochem. 6: 35-38.

Borden, J. H. and Slater, C. E. (1968) Induction of flight muscle
degeneration by synthetic juvenile hormone in *Ips confusus*
(Coleoptera: Scolytidae). Z. Vergl. Physiol. 61: 366-368.

Bowers, W. S. and Aldrich, J. R. (1980) *In vivo* inactivation of
denervated corpora allata by precocene II in the bug
Oncopeltus fusciatus. Experientia 36: 362-364.

Bowers, W. S. and Martinez-Pardo, R. (1977) Antiallatotropins: Inhibition of corpus allatum development. Science (abstr.) 197: 1369-1371.

Bowers, W. S., Ohta, T., Cleere, J. H. and Marsella, P. A. (1976) Discovery of antijuvenile hormones in plants. Science (Wash.) 193: 542-548.

Brett, C. H. (1947) Interrelated effects of food, temperature, and humidity on the development of the lesser migratory grasshopper, *Melanoplus mexicanus mexicanus* (Saussure). Okla. agric. Exp. Sta. No. T-26. 50 pp.

Brosemer, R. W., Vogell, W., and Bucher, Th. (1963) Morphologische und enzymatische Muster bei der Entwicklung indirekter Flungmuskeln von *Locusta migratoria*. Biochem. Z. 338: 854-910. (In German)

Buckell, E. R. (1922) A list of the orthoptera and dermaptera recorded from British Columbia prior to the year 1922 with annotations. Proc. Ent. Soc. of B.C. 20: 9-41.

Bursell, E. (1973) Development of mitochondrial and contractile components of the flight muscle in adult tsetse flies *Glossina morsitans*. J. Insect Physiol. 19: 1079-1086.

Cassier, P. (1965) Le comportement phototropique du criquet migrateur (*Locusta migratoria migratoriodes* R&F): bases sensorielles et endocrines. Annls. Sci. Nat. (Zool.) 7: 213-358.

Cassier, P. (1966) L'activité des corps allates et la reporduction du criquet migrateur africain *Locusta migratoria migratorioides*. Bull. Soc. Zool. Fr. 91: 133-148.

Cassier, P. and Delorme-Joulie, C. (1976) (The imaginal differentiation of the integument of *Schistocerca gregaria* Forsk. III Phase differences and their determination.) Insectes Sociaux <u>23</u>: 179-198.

Chapman, R. F. (1969) The Insects, Structure andFunction. C. Tinling and Co. Ltd., London. pp. 231-241, 406-409, 705-711.

Chapman, R. F., Cook, A. G., Mitchell, G. A. and Page, W. W. (1978) Wing dimorphism and flight in *Zonocerus variegatus* (L.) (Orthoptera, Acridoidea). Bull. Ent. Res. <u>68</u>: 229-242.

Chenevert, R., Paquin, R. and Perron, J. M. (1979) (Aging action of Precocene I on *Schistocerca gregaria*.) Nat. Can. <u>105</u>: 425-427.

Chiharo, C. J. and Fristrom, J. W. (1973) Effects and interactions of juvenile hormone and β-ecdysone on *Drosophila* imaginal discs cultured *in vitro*. Dev. Biol. <u>35</u>: 36-46.

Chudakova, I. V. and Bocharova-Messner, O. M. (1968a) Endocrine regulation of the condition of the wing musculature in the imago of the house cricket, *Acheta domestica*. Proc. Acad. Sci. USSR <u>179</u>: 157-159.

Chudakova, I. V. and Bocharova-Messner, O. M. (1968b) Endocrine regulation of the condition of the wing musculature in the adults of the house cricket, *Acheta domestica*. Proc. Acad. Sci. USSR <u>179</u>: 489-492.

Chudakova, I. V., Bocharova-Messner, O. M. and Novak, V. (1976) Action of juvenile hormone analogues on the development of the skeleton-muscle system of flight apparatus in *Acheta domestica*, L. (Insecta; Orthoptera). Gen. Comp. Endocrinol. 29(2): 295-296.

Chudakova, I. V. and Gutmann, E. (1978) Developmental changes of succinate dehydrogenase, ATPase and acid phosphatase activity in flight muscles of the normal and allatectomized adult cricket, *Acheta domestica* (Orthoptera). Zool. Jb. Physiol. Bd. 82: S1-15.

Davey, J. T. (1959) The African migratory locust (*Locusta migratoria migratoriodes* Rch. & Frm., Orthoptera) in the Central Niger Delta. Part 2. The Ecology of *Locusta* in the semi-arid lands and seasonal movements of populations. *Locusta* 7: 180 pp.

Doane, W. W. (1973) Role of hormones in insect development. pp. 291-479. *In* S. J. Counce and C. H. Waddington, Developmental Systems: Insects. Vol. 2. Academic Press, New York.

Edwards, F. J. (1970) Endocrine control of flight muscle histolysis in *Dysdercus intermedius*. J. Insect Physiol. 16: 2027-2033.

Elliott, R. H. and Gillott, C. (1976) Histological changes in the ovary in relation to yolk deposition, allatectomy, and destruction of the median neurosecretory cells in *Melanoplus sanguinipes*. Can. J. Zool. 54: 185-192.

Elliott, R. H. and Gillott, C. (1977) Changes in protein concentration and volume of the haemolymph in relation to yolk deposition, ovariectomy, allatectomy, and cautery of the median neurosecretory cells in *Melanoplus sanguinipes*. Can. J. Zool. 55: 97-103.

Elliott, R. H. and Gillott, C. (1978) The neuro-endocrine control of protein metabolism in the migratory grasshopper, *Melanoplus sanguinipes*. J. Insect Physiol. 24: 119-126.

Elliott, R. H. and Gillott, C. (1979) An electrophoretic study of proteins of the ovary, fat body, and haemolymph in the migratory grasshopper, *Melanoplus sanguinipes*. J. Insect Physiol. 25: 405-410.

Faure, J. C. (1933) The phases of the Rocky Mountain locust *Melanoplus mexicanus* (Saussure). J. Econ. Ent. 26: 706-718.

Fridman-Cohen, S. and Pener, M. P. (1980) Precocenes induce effect of juvenile hormone excess in *Locusta migratoria*. Nature (Lond.) 286: 711-713.

Friedel and Gillott (1976a) Male accessory gland substance of *Melanoplus sanguinipes*: an oviposition stimulant under the control of the corpus allatum. J. Insect Physiol. 22: 489-495.

Friedel, T. and Gillott, C. (1976b) Extraglandular synthesis of accessory reproductive gland components in male *Melanoplus sanguinipes*. J. Insect Physiol. 22: 1309-1314.

Friedel, T. and Gilliott, C. (1977) Contributions of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. J. Insect Physiol. 23: 145-151.

Fuzeau-Braesh, S. and Nicolas, G. (1970) The influence of carbon dioxide on some phase polymorphic characters in *Locusta migratoria* (L.). Proc. Int. Study Conf. Curr. Fut. Prob. Acrid., London. pp. 93-98.

Gilbert, L. J. and King, D. S. (1973) Physiology of growth and development. pp. 249-370. *In* M. Rockstein, The Physiology of Insecta, Vol. 1 (2nd Ed.). Academic Press, New York.

Gillett, S. D. (1978) Environment determinants of phase polymorphism of the desert locust, *Schistocerca gregaria* (Forsk.) reared crowded. Acrida 7: 267-288.

Gillett, S. D. and Phillips, M. L. (1977) Faeces as a source of a locust gregarisation stimulus. Effects on social aggregation and on cuticular color of nymphs of the desert locust, *Schistocerca gregaria* (Forsk.). Acrida 6: 279-286.

Gillott, C. and Dogra, G. S. (1972) Neurosecretory cell and corpus allatum activity during production of successive egg batches in virgin *Melanoplus sanguinipes* (Fab.). Gen. Comp. Endocrinol. 18: 126-132.

Gillott, C. and Elliott, R. H. (1976) Reproductive growth in normal, allatectomized, median-neurosecretory cell-cauterized, and ovariectomized females of *Melanoplus sanguinipes*. Can. J. Zool. 54: 162-171.

Gilliott, C. and Friedel, T. (1976) Development of accessory reproductive glands and its control by the corpus allatum in adult male *Melanoplus sanguinipes*. J. Insect Physiol. 22: 365-372.

Gurney, A. B. (1952) Grasshopper Glacier of Montana and its
relation to long-distance flights of grasshoppers. Rep.
Smithson. Instn. pp. 305-326.

Helfer, J. R. (153) How to Know the Grasshoppers, Cockroaches and
Their Allies. Wm. C. Brown, Dubuque, Iowa.

Hewitt, G. B. (1977) Review of forage losses caused by rangeland
grasshoppers. U.S.D.A. Misc. Pub. 1348. 24 pp.

Hill, D. S. (1975) Agricultural Insect Pests of the Tropics and
Their Control. Cambridge Univ. Press, New York. pp. 113-117.

Hill, L., Luntz, A. J. and Steele, P. A. (1968) The relationship
between somatic growth, ovarian growth, and feeding activity
in the adult desert locust. J. Insect Physiol. 14: 1-20.

Huffaker, C. B. and Messenger, P. S. (1976) Theory and Practice of
Biological Control. Academic Press, New York. pp. 399-400.

Huibregetse-Minderhoud, L., Van Den Hondel-Franken, M. A. M.,
van der Kerk-Van Hoof, A. C., Biessels, H. W. A.,
Salemink, C. A., Van der Horst, D. J. and Beenakkers, A. M. Th.
(1980) Quantitative determination of the juvenile hormones
in the haemolymph of *Locusta migratoria* during normal
development and after implantation of corpora allata. J. Insect
Physiol. (In press)

Hunter-Jones, P. (1958) Laboratory studies on the inheritance of
phase characteristics in locusts. Anit-Locust Bull.
29. 32 pp.

Johnson, C. G. (1969) Migration and Dispersal of Insects by Flight. Methuen, London. pp. 188-235.

Johnson, R. A. and Hill, L. (1973a) The activity of the corpora allata in the fourth and fifth instars of the migratory locust. J. Insect Physiol. 19: 1921-1932.

Johnson, R. A. and Hill, L. (1973b) Quantitative studies on the activity of the corpora allata in adult male *Locusta* and *Schistocerca*. J. Insect Physiol. 19: 2459-2467.

Johnson, R. A. and Hill, L. (1975) Activity of the corpora allata in the adult female migratory locust. J. Insect. Physiol. 21: 1517-1519.

Joly, L. (1960) Fonctions des corpora allata chez *Locusta migratoria* (L.) Thèse, Strasbourg.

Joly, P. and Meyer, A. S. (1970) (Action of juvenile hormone on *Locusta migratoria* (Orth., Acrididae) in the gregarious phase.) Arch. Zool. Exp. Gen. 11: 51-63.

Kelly, T. J. and Fuchs, M. S. (1978) Precocene is not a specific antigonadotropic agent in adult female *Aedes aegypti*. Phys. Ent. 3: 297-301.

Kennedy, J. S. (1961) Continuous polymorphism in locusts. Symp. Insect Polymorphism, Ent. Soc. Lond., pp. 80-90.

Kruse Pedersen, L.-E. (1978) Effects of anti-juvenile hormone (precocene I) on the development of *Locusta migratoria* L. Gen. Comp. Endocrinol. 36: 502-509.

Loher, W. (1961) The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. Proc. R. Soc. (B) 153: 380-397.

Lowry, O. H., Rosebrough, N. J., Lewis Farr, A. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Luscher, M., Buhlmann, G. and Wyss-Huber, M. (1971) Juvenile hormone and protein synthesis in adult female cockroaches. Bull. Soc. Ent. Suisse 44: 197-205.

Masner, P., Bouers, W. S., Kalin, M. and Muhle, T. (1979) Effect of prococene II in the endocrine regulation of development and reproduction in the bug, *Oncopeltus fasciatus*. Gen. Comp. Endocrinol. 37: 156-166.

McCaffery, A. R. and Page, W. W. (1978) Factors influencing the production of long-winged *Zonocerus variegatus*. J. Insect Physiol. 24: 465-472.

Metcalf, C. L. and Flint, W. P. (1962) Destructive and Useful Insects. (4th Ed.) McGraw-Hill, New York. pp. 461-471.

Miall, R. C. and Mordue, W. (1980) Procecene II has juvenile hormone effects in fifth instar *Locusta migratoria*. J. Insect Physiol. 26: 361-364.

Muller, P. J., Masner, P., Kalin, M. and Bowers, W. S. (1979) *In vitro* inactivation of corpora allata of the bug *Oncopeltus fasciatus* by precocene II. Experientia 35: 704-705.

Nemec, V. (1970) Natural and synthetic materials with insect hormone activity. 7. Juvenile activity of the farnesane-type compounds on *Locusta migratoria* L. and *Schistocerca gregaria* (forsk.). Life Sci. <u>9</u>: 821-831.

Nolte, D. J. (1976) Phase differences between *Locusta* strains. Acrida <u>6</u>: 75-83.

Nolte, D. J. (1978) Eye pigmentation as a locust phase characteristic. Acrida <u>7</u>: 231-241.

Oberlander, H. and Silhacek, D. L. (1976) Action of juvenile hormone on imaginal wing discs of the Indian meal moth. pp. 220-223. *In* L. Gilbert, The Juvenile Hormones. Plenum Press, New York.

Ohta, T., Kuhr, R. J. and Bowers, W. S. (1977) Radiosynthesis and metabolism of the insect antijuvenile hormone, precocene II. J. Agric. Food Chem. <u>25</u>: 478-481.

Ordish, G. (1976) The Constant Pest. Scribner's, New York. p. 57-61, 174-176.

Panar, L. C. and Nair, K. K. (1975) Cytochemistry of the differentiating flight muscles of the desert locust *Schistocerca gregaria*. Histochem. 45: 129-141.

Parker, J. R. and Connin, R. V. (1964) Grasshoppers; their habits and damage. U.S.D.A. Agric. Info. Bull. No. 287. 28 pp.

Parker, J. R., Newton, R. C. and Shotwell, R. L. (1955) Observations on the mass flights and other activities of the Migratory Grasshopper. Tech. Bull. U.S. Dept. Agric. No. 1109. 46 pp.

Patel, N. and Madhavan, K. (1969) Effects of hormones on RNA and protein synthesis in the imaginal wing discs of the ricini silkworms. J. Insect Physiol. 15: 2141-2150.

Pener, M. P. (1967) Effects of allatectomy and sectioning of the nerves of the corpora allata on oocyte growth, male sexual behavior and color change in adults of *Schistocerca gregaria*. J. Insect Physiol. 13: 665-684.

Pener, M. P., Orshan, L. and DeWilde, J. (1978) Precocene II causes atrophy of corpora allata in *Locusta migratoria*. Nature 272 (Land.): 350-353.

Pfeiffer, I. W. (1945) Effect of the corpora allata in the metabolism of adult female grasshoppers. J. Exp. Biol. 99: 183-233.

Pickford, R. (1958) Observations on the reproductive potential of *Melanoplus bilituratus* (Wlk.) reared on different food plants in the laboratory. Can. Ent. 90: 483-485.

Pickford, R. and Mukerji, M. K. (1974) Assessment of loss in yield of wheat caused by the migratory grasshopper, *Melanoplus sanguinipes*. Can. Ent. 106: 1219-1226.

Pickford, R. and Randell, R. L. (1969) A non-diapause strain of the migratory grasshopper, *Melanoplus sanguinipes*. Can. Ent. 101: 894-896.

Poels, C. L. M. and Beenakkers, A. M. T. (1969) The effects of corpus allatum implantation on the development of flight muscle and fat body in *Locusta migratoria*. Ent. Exp. Appl. 12: 312-324.

Pound, J. M. and Oliver, J. H. (1979) Juvenile hormone: evidence of its role in reproduction of ticks. Science (Wash.)206: 355-357.

Pratt, G. E. and Bowers, W. S. (1977) Precocene II inhibits juvenile hormone biosynthesis by cockroach corpora allata *in vitro*. Nature (Lond.)265: 548-550.

Pratt, G. E., Jennings, R. C., Hamnett, A. F. and Brooks, G. T. (1980) Lethal metabolism of precocene I to a reactive epoxide by locust corpora allata. Nature (Lond.)284: 320-323.

Rankin, M. A. (1980) Effects of precocene I and II on flight behavior in *Oncopeltus fasciatus*, the migratory milkweed bug. J. Insect Physiol. 26: 67-73.

Riegert, P. W. (1962) Flight of grasshoppers in the laboratory. Nature (Lond.)194: 1298-1299.

Riegert, P. W. (1965) Effects of grouping, pairing, and mating on the bionomics of *Melanoplus bilituratus* (Walker). Can. Ent. 97: 1046-1051.

Riegert, P. W. (1968) A history of grasshopper abundance surveys and forecasts of outbreaks in Saskatchewan. Mem. Ent. Soc. Can., No. 52. 99 pp.

Rockstein, M. and Miquel, J. (1973) Aging in insects. pp. 371-478. *In* M. Rockstein, The Physiology of Insecta, Vol. 1 (2nd Ed.). Academic Press, New York.

Rowell, C. H. F. (1967) Corpus allatum implantation and green/brown polymorphism in three African grasshoppers. J. Insect Physiol. 13: 1401-1412.

Sahota, T. S. (1975) Effect of juvenile hormone on acid phosphatases in the degenerating flight muscles of the Douglas-fir beetle, *Dendroctonus pseudotsugae*. J. Insect Physiol. 21: 471-478.

Sahota, T. S. and Farris, S. H. (1980) Inhibition of flight muscle degneration by precocene II in the spruce bark bettle, *Dendroctonus rufipennis* (Kirby) (Coleoptera:Scolytidae). Can. J. Zool. 58: 378-381.

Schacterle, G. R. and Pollack, R. L. (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Biochem. 51: 654-655.

Schooneveld, H. (1979) Precocene-induced collapse and resorption of corpora allata in nymphs of *Locusta migratoria*. Experientia 35(3): 363-364.

Sehnal, F. (1971) Juvenile hormone action and insect growth rate. Endocrinol. Epx. 5: 29-33.

Shirk, P. D., Bhaskaran, G. and Roller, H. (1980) The transfer of juvenile hormone from male to female during mating in the *Cecropia* silkworm. Experientia 36: 682-683.

Staal, G. B. (1961) Studies on the physiology of phase induction in *Locusta migratoria migratoriodes* R. and F. Pub. Fds. Landb. Export Bur., No. 40 (Thesis, Wageningen).

Staal, G. B. (1967) Plants as a source of insect hormones. Proc. K. Ned. Akad. Wet. (c) 70: 409-418.

Staal, G. B. (1975) Insect growth regulators with juvenile hormone activity. Ann. Rev. Ent. 20: 417-460.

Stegwee, D., Kimmel, E. C., De Boer, J. A. and Henstra, S. (1963) Hormonal control of reversible degeneration of flight muscle in the Colorado potato beetle *Leptinotarsa decimlineata* Say. J. Cell Biol. 19: 519.

Tarrant, C. A. and Cupp, E. W. (1978) Morphogenetic effects of precocene II on the immature stages of *Rhodnium prolixus*. Trans. R. S. Trop. Med. and Hyg. 72(6): 666-668.

Tobe, S. S. and Pratt, G. E. (1976) The synthetic activity and glandular volume of the corpus allatum during ovarian maturation in the desert locust *Schistocerca gregaria*. Life Sci. 17: 417-422.

Unnithan, G. C. and Nair, K. K. (1977) Ultrastructure of juvenile hormone-induced degenerating flight muscles in a bark beetle *Ips paraconfusus*. Cell Tiss. Res. 185: 481-490.

Unnithan, G. C. and Nair, K. K. (1979) The influence of corpus allatum activity on the susceptibility of *Oncopeltus fasciatus* to precocene 1, 2, 3. Ann. Ent. Soc. Am. 72: 38-40.

Unnithan, G. C., Nair, K. K. and Syed, A. (1980) Precocene-induced metamorphosis in the desert locust *Schistercerca gregaria*. Experientia 36: 135-136.

Uvarov, B. (1966) Grasshoppers and Locusts, Vol. 1. Cambridge University Press, London. pp. 84, 288-289.

Uvarov, B. (1977) Grasshoppers and Locusts, Vol. 2. Cambridge University Press, London. pp. 524-531.

Van den Hondel-Franken, M. A. M., Van Den Broek, A. Th. M., and Beenakkers, A. M. Th. (1980) Flight muscle development in *Locusta migratoria*: effects of implantation of corpora allata on the attainment of metabolic enzyme activities. Gen. Comp. Endocrinol. (in press).

van Marrewijk, W. J., Schrikker, A. E., and Beenakkers, A. M. (1980) Contents of nucleic and amino acids and rate of protein synthesis in developing flight muscles of *Locusta migratoria*. Comp. Biochem. Physiol. 65B: 251-257.

Verma, K. B. (1981) Roles of juvenile hormone in the green peach aphid, *Myzus persicae* Sulzer (homoptera:Aphididae). Thesis. U.B.C., Vancouver, B.C.

Vogel, W., Masner, P., Graf, O. and Dorn, S. (1978) Types of response of insects on treatment with juvenile hormone active insect growth regulators. Experientia 35: 1254-1256.

Williams, C. M. (1961) The juvenile hormone. II. Its role in the endocrine control of molting, pupation and adult development in the *Cecropia* silkworm. Biol. Bull. (Woods Hole) 121: 572-785.

Willis, H. R.   (1939)   Painting for determination of grasshopper

flights.   J. Econ. Ent. 32: 401-403.

Willis, J. H.   (1974)   Morphogenetic action of insect hormones.

Ann. Rev. Ent. 19: 91-116.

APPENDIX 1: Means and standard errors for growth measurements in
early adult *M. sanguinipes*

Age (days)	Total body fresh weight (mg)	Tibia length (mm)	Tegmina and wing length (mm)	Head width (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)
Males							
1	230.8±23.7	10.6±0.4	19.0±0.7	4.0±0.1	4.2±0.5	5.1±0.5	6.0±0.4
2	286.1±23.3	10.4±0.2	19.0±0.5	3.9±0.1	4.8±0.5	5.8±0.9	7.2±0.7
3	337.5±16.3	10.2±0.2	19.2±0.4	3.9±0.1	6.0±0.5	8.3±1.6	9.4±0.7
4	340.4±12.3	10.2±0.2	19.0±0.4	3.9±0.1	8.8±0.5	9.9±0.6	11.7±1.0
5	387.4±27.3	10.8±0.3	19.3±0.6	4.0±0.1	10.3±1.1	11.7±1.6	14.1±1.5
6	349.5±13.2	10.2±0.1	19.3±0.3	3.9±0.0	10.6±0.5	10.8±1.0	14.1±1.0
7	349.4±14.5	10.3±0.3	19.3±0.8	3.9±0.1	10.6±0.6	8.8±1.6	13.2±1.3
8	340.2±14.0	10.1±0.2	18.9±0.8	3.9±0.1	9.7±0.4	8.1±1.3	12.7±1.0
9	327.6±6.5	10.0±0.1	19.2±0.3	3.8±0.0	10.2±0.5	8.5±0.5	12.8±0.6
Females							
1	219.9±8.9	11.1±0.1	19.2±0.6	3.9±0.1	1.6±0.1	5.9±0.3	6.1±0.5
2	328.2±11.2	11.3±0.2	19.7±0.4	4.3±0.1	1.7±0.2	9.1±0.7	8.3±0.7
3	383.4±26.9	11.2±0.3	20.1±0.6	4.2±0.1	2.4±0.3	13.5±2.1	11.0±1.3
4	434.1±17.6	11.7±0.2	20.7±0.2	4.3±0.1	7.5±1.5	24.3±2.1	15.3±0.7
5	475.8±34.8	11.4±0.2	20.0±0.7	4.2±0.1	26.6±1.6	24.8±3.5	17.5±2.0
6	471.1±17.7	11.1±0.2	19.9±0.4	4.2±0.1	35.0±1.6	17.8±1.3	15.0±1.2
7	509.1±24.9	11.8±0.3	20.6±0.3	4.3±0.1	40.2±6.9	18.5±1.4	16.3±2.1
8	504.8±32.9	11.9±0.2	20.6±0.6	4.3±0.1	45.6±6.3	16.0±2.0	14.8±1.4
9	428.7±18.4	11.4±0.2	20.0±0.2	4.1±0.1	29.1±2.4	16.0±1.8	14.0±0.8

APPENDIX 2: Means and standard errors for normal
total body fresh weight in early adult
M. sanguinipes (N = 4)

Age (days)	Mean total body fresh weight (mg)	Standard error
Males		
1	228.4	11.6
2	239.0	8.8
3	276.1	18.6
4	288.5	16.7
5	296.3	16.9
8	288.0	15.7
9	325.0	18.7
Females		
1	263.6	0.8
2	292.1	1.7
3	316.2	12.3
4	249.7	2.0
5	381.9	11.2
8	421.6	28.8
9	432.0	13.5

APPENDIX 3: Means and standard errors for flight muscle dry weight and protein content in normal early adult *M. sanguinipes* (N = 2 to 3)

| | Males | | | Females | |
| | Mean flight muscle dry weight (mg) | Mean flight muscle protein content (mg) | | Mean flight muscle dry weight (mg) | Mean flight muscle protein content (mg) |
Age (days)			Age (days)		
Trial 1[a]			Trial 1[c]		
2	8.6±0.6	3.9±0.2	2	7.9±1.4	3.9±0.2
3	10.2±1.2	7.5±1.1	3	12.6±1.7	6.3±0.6
4	11.4±0.6	6.4±0.4	4	14.8±1.1	8.5±1.4
5	11.4±1.8	7.2±1.1	5	16.2±2.8	8.2±1.2
6	13.8±1.2	7.7±1.4	6	14.3±0.5	8.4±0.6
7	10.3±0.5	6.7±0.5	7	12.3±0.6	6.6±0.7
8	11.8±0.1	5.5±0.8	8	13.9±2.2	8.6±0.4
9	12.0±0.6	7.4±0.9	9	12.3±0.1	6.6±0.9
Trial 2[b]			Trial 2[d]		
1	6.0±0.4	3.7±0.4	1	6.1±0.5	3.5±0.4
2	5.9±0.3	3.6±0.0	2	8.7±0.8	7.1±0.7
3	8.5±0.5	5.5±0.5	3	9.4±1.8	5.8±0.8
4	12.0±2.2	7.7±1.5	4	15.8±1.0	10.4±1.3
5	16.9±0.6	10.4±0.6	5	18.7±3.1	12.1±1.6
6	14.4±1.7	9.5±1.0	6	15.7±2.3	9.7±1.5
7	16.0±0.8	10.6±0.3	7	20.3±2.3	11.6±2.0
8	13.3±1.6	7.5±0.8	8	15.8±1.9	10.4±0.5
9	13.5±0.9	8.5±0.5	9	15.6±0.8	9.0±0.6

a   not significant

b   D4, D6 >D0, D1, D2; D5>D1, D0; D8>D1

c. D4, D5>D2

d   D4, D6>D1; D6>D2

APPENDIX 4: Mean (± S.D.) body parameters of 5 day-old adult males treated with 0.05 µg R-20458 at various intervals during the fifth instar

MALES

Trial	Day of application	Total body fresh weight (mg)	Tibia length (mm)	Head width (mm)	Tegmina width (mm)	Wing length (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)
1	1	351.3±22.4	10.8±0.3	4.0±0.2	9.8±1.2	10.0±1.8	8.1±1.6	13.7±2.5	10.7±1.4
	3	304.1±43.9	11.0±0.3	3.9±0.2	13.7±2.1	11.1±1.5	6.5±1.6	6.5±0.9	6.9±1.1
	4	294.3±29.2	10.0±0.5	3.6±0.3	17.1±0.7	17.1±0.7	8.1±0.7	8.3±2.7	10.1±2.3
	5	361.2±30.5	11.1±0.5	4.0±0.1	16.4±1.3	11.8±1.0	8.4±1.3	8.0±3.2	9.7±0.6
	6	307.3±24.8	10.7±0.1	3.8±0.1	18.9±0.4	18.6±0.7	8.4±0.3	6.2±2.3	11.6±1.0
	Grand mean	323.6±38.5	10.7±0.5	3.9±0.2	15.2±3.5	13.7±3.7	7.9±1.3	8.5±3.5	9.8±2.0
2	1	318.6±22.7	10.6±0.0	3.9±0.1	10.4±1.6	10.4±1.6	11.7±1.8	5.7±1.5	12.0±0.7
	2	323.2±51.4	10.6±0.8	3.8±0.2	10.5±2.2	10.5±2.2	8.6±1.7	8.7±2.1	8.0±4.3
	4	340.0±30.4	10.6±0.4	3.4±0.2	15.6±0.6	14.3±0.6	8.9±1.7	10.4±2.6	9.0±1.4
	4 1/2	304.4±19.5	10.3±0.5	3.7±0.2	18.2±0.1	18.2±0.1	7.8±1.1	10.6±0.9	9.6±1.8
	4 3/4	341.5±27.9	11.1±0.2	3.9±0.2	18.3±0.8	15.9±1.8	8.6±0.2	10.7±2.4	9.4±1.9
	5	345.1±45.0	10.8±0.6	3.8±0.1	19.0±1.4	19.0±1.4	9.3±0.1	11.9±1.6	11.6±2.9
	Grand mean	327.8±31.7	10.7±0.5	3.8±0.2	15.1±3.9	14.5±3.6	9.2±1.7	9.5±2.6	9.8±2.5

APPENDIX 5: Mean (± S.D.) body parameters of 5 day-old adult females treated with 0.05 μg R-20458 at various intervals during the fifth instar

FEMALES

Trial	Day of application	Total body fresh weight (mg)	Tibia length (mm)	Head width (mm)	Tegmina width (mm)	Wing length (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)
1	1	491.5±29.2	12.6±0.6	4.4±0.2	12.1±2.3	12.1±1.9	2.5±0.4	28.4±4.4	11.1±1.8
	3	396.9±90.9	11.9±0.4	4.2±0.3	14.9±3.9	12.3±1.7	4.1±1.5	14.6±3.7	10.2±3.5
	4	478.8±19.5	11.5±0.3	4.2±0.1	20.0±0.5	20.0±0.5	29.8±3.0	23.4±3.9	15.5±1.9
	5	383.7±80.3	11.4±0.0	4.1±0.2	14.6±2.4	12.6±1.6	6.7±2.4	19.9±7.0	11.0±2.6
	6	408.1±22.4	11.2±0.3	4.0±0.1	18.6±0.5	18.2±1.1	24.3±2.1	15.0±1.8	12.1±0.9
	Grand mean	431.8±66.8	11.7±0.6	4.2±0.2	16.0±3.6	15.1±3.7	13.5±11.9	20.2±6.6	12.0±2.7
2	1	420.1±56.7	11.1±0.6	4.2±0.1	13.0±2.8	11.9±1.6	51.8±22.2	7.1±2.0	2.9±1.2
	2	-	-	-	-	-	-	-	-
	4	452.5±69.4	11.5±0.5	4.2±0.2	16.2±0.8	14.4±0.7	27.5±4.4	23.5±4.4	12.0±1.0
	4 1/2	467.6±48.9	12.0±0.3	4.2±0.1	19.5±0.4	19.2±0.4	35.1±13.7	20.5±2.5	12.0±1.0
	4 3/4	418.8±42.2	11.8±0.2	4.2±0.1	18.5±0.8	16.5±0.8	28.4±6.2	19.7±1.2	12.1±0.9
	5	483.8±8.1	12.0±0.2	4.2±0.1	20.3±0.1	19.7±0.6	26.6±10.5	23.6±3.2	14.2±2.2
	Grand mean	448.6±47.0	11.7±0.4	4.2±0.1	17.5±2.9	16.4±3.1	33.9±14.7	18.8±6.7	12.0±1.8

APPENDIX 6: Raw data: Precocene-treated adultoids ± a later treatment with JHA

		Body Measurements						
Treatment	Insect fresh weight (mg)	Tibia length (mm)	Tegmina length (mm)	Head width (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)	Wing length (mm)
FEMALES								
Control	212.8	8.2	6.3	3.4	24.7	5.5	6.3	6.3
(Precocene only)	168.1	8.0	4.7	3.3	2.2	17.6	7.3	4.7
Adultoids	156.5	8.0	5.2	3.2	1.9	14.2	6.7	5.2
JHA-treated	188.8	8.3	7.3	3.4	21.6	6.4	4.7	7.3
Adultoids	186.9	8.3	7.5	3.3	14.8	7.9	6.3	7.5
	194.7	8.5	5.8	3.2	16.2	11.6	6.0	5.8
MALES								
Control	161.7	8.1	7.5	3.1	5.7	7.0	5.7	7.5
(Precocene only)	157.2	8.2	5.5	3.2	4.2	6.0	6.3	5.5
Adultoids	162.0	8.0	6.6	3.1	5.4	6.2	6.4	6.6
JHA-treated	129.7	7.9	5.7	3.1	3.7	1.4	3.4	5.7
Adultoids	154.7	8.1	7.1	3.2	5.7.	4.4	6.1	7.8
	144.5	8.0	5.7	3.2	4.8	4.7	6.0	5.7

APPENDIX 7: Raw data: reversing precocious matamorphosis by timed applications of JHA to fourth
instar precocene-treated *M. sanguinipes*

Treatment	Insect fresh weight (mg)	Tibia length (mm)	Tegmina length (mm)	Wing length (mg)	Head width (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)	Resulting type
A. MALES									
Control (Precocene only)	193.6	8.5	5.9	5.9	3.4	6.3	9.5	7.1	Adultoid
Control	for more control insects, see Table 21								
JHA applied day 4	295.9	9.7	18.6	17.8	3.7	7.7	11.7	9.6	
	260.6	9.7	14.0	12.4	3.6	7.8	4.4	6.5	Normal-looking adults
	253.6	9.3	16.7	15.5	3.4	5.8	6.8	6.3	
JHA applied day 5	133.9	10.5	17.7	17.7	3.6	3.0	0.5	2.7	
JHA applied day 6	302.6	9.9	18.4	16.5	3.8	8.3	7.2	9.2	Reproductive adults
	321.3	10.7	18.3	18.3	3.6	10.2	8.3	7.4	
	143.9	7.8	6.3	6.3	2.9	4.3	4.0	3.7	Adultoid
B. FEMALES									
Control (Precocene only)	161.8	7.9	6.5	6.5	3.2	1.8	14.9	6.8	
	185.0	8.2	6.0	6.0	3.3	2.7	14.1	7.6	
	217.9	8.6	7.5	7.5	3.6	1.6	16.2	8.0	Adultoids
	164.9	8.1	7.0	7.0	3.2	1.6	10.7	7.3	
	164.2	7.7	6.2	6.2	3.2	2.2	15.4	6.2	
JHA added day 4	297.1	9.8	16.3	13.8	3.6	1.8	14.0	8.9	
	324.6	9.7	16.2	14.5	3.8	11.7	9.2	7.4	Normal-looking adults
	341.2	11.3	18.4	15.5	4.0	2.2	21.8	9.1	
JHA added day 5	261.2	10.5	17.2	17.2	3.7	2.2	15.2	9.5	
JHA added day 6	371.8	11.4	19.7	19.0	4.1	42.9	13.4	11.1	Reproductive adults
	427.2	10.9	18.7	18.0	4.0	38.7	11.4	12.2	
	185.2	9.9	6.2	6.2	3.2	1.8	14.2	5.7	Adultoid

APPENDIX 8: The effect of DMSO (1 µl) application to fourth instar nymphs on 6-day-old adult body measurements

Treatment	Sex	Fresh body weight (mg)	Tibia length (mm)	Tegmina and wing length (mm)	Head width (mm)	Gonad dry weight (mg)	Fat body dry weight (mg)	Flight muscle dry weight (mg)
Untreated	M	343.3	10.7	20.4	3.8	9.3	9.1	12.0
	M	391.3	10.9	20.5	3.8	11.7	12.4	12.1
	F	386.1	11.1	19.7	3.9	15.3	17.7	11.2
DMSO	M	394.0	11.2	20.2	4.0	10.8	13.8	12.5
(1 µl)	F	399.5	11.3	21.6	4.1	5.9	16.8	14.9
	F	328.5	10.8	19.6	3.8	6.1	14.2	9.3
	F	476.2	11.0	20.1	4.3	7.6	23.3	13.2