ENDOCRINE AND CERTAIN ENVIRONMENTAL FACTORS INVOLVED IN POLYMORPH DIFFERENTIATION AND CONTROL OF THE APHID *Macrosiphum rosaeiformis* (DAS) HOMOPTERA: APHIDIDAE

ABSTRACT

SUDHANYA RAY HAJONG

DEPARTMENT OF ZOOLOGY
NORTH-EASTERN HILL UNIVERSITY

Thesis

ABSTRACT

The present study was conducted to study some of the endocrine and environmental factor responsible for the phenomenon of polymorph differentiation in the aphid *Macrosiphum rosaeiformis* (Das). Apart from it, attempt was also made to evaluate the role of the anti-allatin Precocenes and pheromone 'Queen substance' in the control of aphids.

The present work is divided into five sections namely:

1. Life cycle and polymorph determination:
2. Juvenile hormone and polymorph differentiation.
3. Juvenile hormone and photoperiod in polymorph differentiation.
4. Neurosecretion.
5. Control of aphids.

1: LIFE CYCLE AND POLYMORPH DETERMINATION:

Observation was made on the occurrence of the various morph forms of the aphid *Macrosiphum rosaeiformis*.

Regular observation on the occurrence of the morphs was done periodically. Morph was identified based on their microscopic characters. The following morphs were observed during the course of this study:-

i. Alate virginoparae
ii. Apterous virginoparae.
iii. Alate gynoparae.
iv. Alate Oviparae
v. Apterous Oviparae.
vi. Intermediate alate/ apterous virginoparae.
vii. Intermediate oviparous/virginoparous
viii. Males.

Reproduction is by means of parthenogenetic means through out the year, but, the occurrence of sexual forms appears during the winter period.No sexual reproduction is however observed . The life cycle appears to be incomplete or intermediate between anholocyclic and holocyclic.

Study was made to observe the effect of photoperiod on the appearance of different morphs. It was observed that under short photoperoid treated individuals give rise to an increased percentage of alate forms. Apart form this, a sequence of morph production id observed with the short photoperiod. Treated parent virginoparous individuals under short photoperiod first give rise to increasing alate forms, these alate forms produced under short photoperiod are the gynoparae that produces the egg laying oviparae. The gynoparae resembles the alate virginoparae superficially but differ in the number of secondary rhinaria in the antennal segment, and differ in the reproductive physiology as well. Apart from these normal morph forms, some intermediate forms were also observed, they exhibit both the features of either the alate/apterous or the virginoparous /oviparous forms.

The effect of photoperiod coupled with low temperature on the differentiation of alate morphs was also studied. It is observed that under a reduced photoperiod the percentage of alate progeny in the treated parents increased, indicating that a reduced photoperiod was responsible for the production of alate morphs under winter short day conditions. Further, the sequence of morph production was also studied. Under a reduced photoperiod and a low temperature the virginoparous forms give rise to the alate gynoparae, these alate gynoparae then give rise to the egg laying oviparae. The occurrence of these virginoparous as well as the egg laying sexual females indicates that *Macrosiphum rosaeiformis* is a Holocyclic species,

However, the lack of any eggs under natural conditions as well as the presence of parthenogenetic reproduction during the entire winter period strongly suggests that it exhibit an incomplete holocyclic mode of life cycle in this region.

2.JUVENILE HORMONE AND POLYMORPH DIFFERENTIATION.

Juvenile hormone has been implicated in the differentiation of polymorph forms in aphids. It is believed that juvenile hormone titer regulated by environmental condition determines the differention of morphs in aphids. However, the exact nature of the role of juvenile hormone in aphids is not yet known. This is due to the size and delicacy of these insects, since techniques like allatactomy cannot be performed, but, with the discovery of the anti-allatin i.e, precocenes, it has become convenient for the aphid physiologist to study the role of the juvenile hormone/corpora allata by using the compound precocene as a chemical allatactomy.

Therefore, the present experiment has been conducted on *Macrosiphum rosaeiformis* (Das) to study the role of juvenile hormone/corpora allata by utilising topical application of juvenile hormone-III and also, by using the anti-allatin precocene-I and II. Further, study was conducted to see the action of the cyclic AMP, which acts as second messenger, in hormone action. This was done with an aim to elicit a response if any, by mimicking the hormone action on morph differentiation.

Application of juvenile hormone –III (Cis-10,11-Epoxy-3,7,11-trimethyl-trans ,trans -2,6-dodecadienoic acid methyl ester) to the pre-alate III and IV instars leads to production of individuals showing different grades of morphological responses. There, is a degeneration of the wings, lack of sclerotisation of pterothorax, lack of marginal sclerites. In case of the third instar nymphs treatment of JH-III also leads to production of supernumerary forms, but, not in case of the IV instar. These individuals shows sign of apterisation by, their morphological similarities with the apterous forms. These results therefore shows that, juvenile hormone is involved in the differentiation of alate morph. Treatment of the anti-allatin precocene-I and II has been found, not to increase the percentage of alate progeny, both in the long day alate and the apterous parent. It indicates that, either, there are factors other than juvenile hormone involved in the determination of alate morphs under long day conditions or there is an apparent juvenile agonistic effect of precocene, which leads to the apparent increased percentage of apterous progeny rather than the alates. Cyclic AMP treatment has been found to have no effect on the morph differentiation.

3.JUVENILE HORMONE AND PHOTOPERIOD IN MORPH DIFFERENTIATION:

The alate gynoparae that are produced by short day photoperiod by the virginoparae are essentially the alate virginoparae that, produce the egg laying oviparae. Study was conducted to see whether, juvenile hormone is involved in its differentiation. When alate and apterous virginoparous parent are treated under short day photoperiod at a low temperature, it is seen that maximum percentage of progeny consists of the alate forms, but when treated with Juvenile hormone-III, the percentage of alate progeny decreases significantly, whereas, the control batch without hormone treatment shows as usual an increased alate progeny. Further, the study on the corpora allata volume and cytoplasm/ nuclear ratio indicates that the corpora allata activity is more in case of the long day forms than in case of the short day forms.This result therefore, suggests that, the alate gynoparae are produced under short day by an apparent diminishing level of juvenile hormone titer in the parent, brought about by the reduced activity of the corpora allata.

4. NEUROSECRETION:

The neurosecretory cells in the brain was mapped by using the technique of selective stain with Paraldehyde fuschin (PAF). After PAF staining,neurosecretory cells attain deep purple coloration depending upon the contents of the perikaryon. Based upon these four groups of neurosecretory cells in the brain was identified, viz, Group I, Group II, Group III and Group IV. The Group I is the most prominent and lies at the region on either side of the protocerebral cleft. It consists of 4-5 cells. The group I cell have been implicated in the photoperiodic regulation of morph differentiation in the aphid *Megoura*. In this present study it was found that the secretory activity as revealed by the amount of stainable nuclear granules in the perikryon of the Group I cells are dependent upon the photoperiod. Under Long day photoperiod the contents of the stainable paraldehyde positive material is less than that of the short day. Further, the corpora allata neurosecretory cells also shows similar activity under short and long day photoperiod.

Thus, these results indicate that, the corpora allata activity is determined by the photoperiod via the neurosecretory cells. This leads to the change in the titer of the juvenile hormone, which ultimately brings about a change in the morph production.

5.APHID CONTROL:

Attempt was made to evaluate the anti-allatin Precocene-I (7-methoxy-2,2-dimethyl chromene) and –II(6,7-dimethoxy-2, 2-dimethylchromene) and the honebee phermone' Queen substance'(9-oxodec-trans-2-enoic acid) as an aphid control agent. Application of precocene-I and II has been found to lead to severe morphological aberrations in the developed insects. The developed insects had severely crumpled wings as well as deformed appendages. They were incapble of flight. The response obtained was dose dependent. Mortality as well as aberrant forms increased with higher doses. 'Queen substance' when applied to the alate and the apterous virginoparous leads to a reduction in the progeny born by the treated parents. Thus, the use of these compounds as a control agent is demonstrated.

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BY

SUDHANYA RAY HAJONG

DEPARTMENT OF ZOOLOGY

SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF
THE DEGREE OF DOCTOR OF PHILOSHOPY IN ZOOLOGY OF
NORTH – EASTERN HILL UNIVERSITY, SHILLONG.

Dedicated to my parents

THE NORTH-EASTERN HILL UNIVERSITY

DECEMBER, 1999.

I, Sudhanya Ray Hajong, hereby declare that the subject matter of this thesis is record of work done by me, and that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge, to anybody else, and that this thesis has not been submitted by me for any research degree in any other University / Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

B. B. P. Gupta
(Head)
Head Deptt. of Zoology
School of Life Sciences
NEHU., Shillong- 22

A. Raghu Varman
20/12/99
(Supervisor)
Prof. A. Raghu Varman
Department of Zoology
North- Eastern Hill University
Shillong- 793 002.

Sudhanya Ray Hajong
(Candidate)

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Sudhanya Ray Hajong

Sudhanya Ray Hajong

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GENERAL INTRODUCTION

INTRODUCTION:

Aphids are small Homopteran insects that feeds on the plant sap and causes serious infliction to plants by devitalising as well as by transmitting a number of virus causing diseases. They exhibit the phenomenon of multiple morphs i.e. polymorphism or polyphenesia. Their life cycle is often complex and consists of several distinct morph forms that appear only during specific environmental conditions. (Hille Ris Lambers, 1966). The phenomenon of the environmental effect namely daylength and temperature on polymorphism in insects has first been demonstrated in aphids way back in 1924 by Marcovitch. A number of workers have since studied the phenomenon of polymorphism in aphids in its various aspects. The term polymorphism essentially means the exhibition of multiple forms of the same individuals of a sex of a species. In entomological terms polymorphism is generally referred to *'any individuals of a species with distinct but dissimilar external characters'* (Hardie and Lees, 1985). In aphids, polymorphism is mainly of the nongenotypic form, in that the variations between individuals are not genotypically different but are of the same genotypic constitution but they arise due to entirely different developmental pathway or routes determined by environmental factors which acts as a signal acting at specific points in development which vary from the zygote to a stage late in the postembryonic development. Genetic polymorphism as in case of the peppered moth, *Biston betularia* is a case of transient polymorphism and the mimicry of female swallowtail, *Papilio dardanus* is a case of balanced polymorphism, these do not differ in their genotype but only in their phenotype. (Hardie and Lees, 1985). In genetic polymorphism environmental selection pressures remove or favour certain genotype within a population, where as in environmental controlled polymorphism this factor forms an essential part of the developmental switching process.

For the present study, we followed the definition as stated by Hardie and Lees (1985); namely *'the occurrence of two or more distinct phenotypes that can be induced in individuals of the same genotype by extrinsic factors'*.

Aphids exhibit non-genetic polymorphism. Non-genetic polymorphism is of two types; Successive and Alternative. Successive polymorphism is the sequential morphological variant observed during the developmental stage of an insect or an individual during the coarse of its life cycle. The change over from a distinct larvae to a pupa and finally to adult in case of Lepidoptera is an example of this type. Alternative polymorphism on the other hand is referred to those morphological forms that arise due to environmental variation. Here, individuals attain a particular morph depending upon the environmental stimuli induced, and further each individual having the inherent ability of the multiple forms. In referring the term 'Polymorphism', we are thus concerned with the monogenetic polymorphism, or more appropriately called the 'Polyphenesia'. Mayr (1963) and Gruneburg (1980) have suggested that the term 'polymorphism' should be used and restricted to the former and proposed the term polyphenism for the latter. Other authors have used the term Oecomorph, Non-genetic and Eco-phenotype to emphasise the environmental component in monogenetic polymorphism (de Wilde, 1975, 1976).

The polymorphic forms of aphids are diverse and often complex, each morph arises under the influence of environmental factors like photoperiodism and temperature (Hille Ris Lambers, 1966; Dixon, 1985). These multiple forms have a survival value for the species, enabling them to escape the rigours of the inclement environmental conditions. The appearance of polymorphic forms in aphids are controlled by the endocrine system in conjunction with the environmental stimuli like photoperiodism, temperature as well as crowding, nutrition etc. (Lees, 1966). Each morph is morphologically and functionally distinct and arises only under the influence of a given factor. Further, during certain times individuals with overlapping or intermediate characters arise (Lees, 1966), which are either reproductively viable or are totally inviable, and are thus at a dead end in their life cycle.

Based upon the polyphenetic forms and mode of their reproduction aphid polymorphism can be divided into two categories (Lees, 1966).

(1) **Alate / apterous dimorphism.**

(2) **Parthenogenetic / Gamic polymorphism.**

Alate / apterous dimorphism is the most prevalent form of polymorphism in aphids, individuals of a large number of species exhibit the apterous or alate conditions depending upon a number of factors. This form of polymorphism is seen in case of both the parthenogenetic as well as the sexual forms depending upon life cycle and environmental conditions. Environmental factors like population density, nutrition, photoperiodism, temperature are usually the main influencing factor in the determination of alate forms in the parthenogenetic forms as has been demonstrated in a number of aphids. In *Megoura* the parthenogenetic forms arise under the influence of crowding under long day photoperiod. Short day photoperiodism and low temperature influence morph determination by producing alate ovipara. (Lees, 1959,60,63,67) In *Aphis fabae*, which is a Holocyclic heteroecious species alate forms are produced by crowding under long day conditions, however under the influence of short day conditions, the apterous virginoparous forms lead to the production of alate virginoparous that are refereed as the Gynopara or the ovipara producer. Other similar host alternating species also have been found to produce alate gynopara under short photoperiodic conditions (Bonnemaison, 1951;Blackman, 1971;Dixon, 1971; Judge, 1968; Schaefers and Judge, 1971). However it has been observed that alate gynoparous forms appear under the conditions of crowding and nutritional change in case of many species of aphids (Bonnemaison, 1968; Judge, 1968; Sethi and Swenson, 1967; Dunn, 1974). The effect of temperature also influences the determination of alates, in general it is observed that low temperature favours alate production and high temperature favours apterous development (Johnson and Birks, 1960;Lamb and White, 1966; Lees, 1967).

The alate and the apterous individuals differ in the presence or absence of adult features, including the sensorimotor apparatus, the wings, specialised pterothorax and placoid sensillae on the antennae and ocelli. There is also difference in the pigmentation pattern. In adult aptera the lack of such features is comparable to the larval stage or an apparent neoteny. This resemblance of the adult aptera with the juvenile forms give rise to the suggestion that the apterous forms give rise due to an apparent decreased level of juvenile hormone.

The regulation of polymorphism in aphid embraces the environment, the endocrine system, which act as the mediator and the morphological character that it controls. Very little is understood how juvenile hormone apart from its role in the control of metamorphosis, is also involved in the dual function of regulation of metamorphosis and also in the determination of the polymorphic forms during the course of an individual's development. (Hardie and Lees, 1985). It is understandable that switching of the alternative pathways in morph determination takes place at a particular time during the developing stages of the insect, when the sensitivity of the insect to juvenile hormone is at its maximum. This sensitive stage lasts only for a short duration during which time the determination of each morph is complete. In *Megoura* sensitivity of the individual to juvenile hormone has been found to be maximum at the third instar stage, and any external topical application of JH brings about a maximum morphological response at this stage. (Lees, 1980).

Initial experiments conducted on developing alate individuals with topical application of Juvenile hormone mimics have yielded unequivocal results. JH mimics when applied to third and fourth alate nymphs were not found to cause total apterisation. Application of potent juvenile hormone compounds brought about some sign of apterisation in the form of retainment of juvenilised features. Based on these it was concluded that the effect produced was a case of juvenilisation rather than apterisation (Lees, 1961; 1966; 1977). The use of juvenoids with higher activity further showed that middle instar alate virginopara develops into near perfect supernumerary alate larvae in the fifth instars, rather than the adult (Lees, 1966 , 1980;

White and Lamb, 1968). The fifth instar adult individuals possessed the larval wing pads and other recognisable larval features.

The activity of the corpora allata has been also correlated with the development of alary polymorphism. In *Brevicoryne brassaciae* it was found that the volume as well as the nuclei of corpora allata were greater in case of apterous larvae than in the alate. In adult however it was reversed (White, 1965,1971). In *Aphis craccivora* a large nuclear diameter in developing apterae than in alate have been attributed to be correlated with the ovarian growth rather than morph determination (Elliot, H., 1955). In the aphid *Megoura viciae* no volume change have been found between adult alate producers and aptera producers (Leckstein and Llewellyn, 1975;Leckstein, 1976). However, positive correlation has been found in case of short day photoperiodic condition. The activity measured by means of the Corpora allata (C.A) volume have been found to be more in case of long day individuals than in case of short day treated individuals (Kats, 1980; Hardie, 1987). In *Myzus persicae* (Sulz) the volume of C.A has been found to be more in case of alate or alate producers. Further the C.A of alate adults increases and that of apterous adults decreases until reproduction commences. Crowding and isolation were found to have no difference on the C.A volume (Srivastava and Verma, 1981).

The alate virginoparae studied are those produced under long day photoperiod and under the influence of crowding. In studying the role or involvement of juvenile hormone in the determination of these forms under long day conditions, there are certain disadvantages. Since the alate progeny in such case cannot be determined with certainty and hence the effect of externally applied hormone on the progeny morph cannot be ascertained unambiguously in relation with the control individuals, where the progeny is highly variable. However, in case of short day induced alate nymphs it is possible to induce in the progeny a maximum percentage of alates forms (Lees, 1975; 77). It is also possible to divert this presumptive alate gynopara into apterous pathway of development by exposing these to long day photoperiod (Lees, 1977: Hardie, 1980b, 1981[illegible]). For instance in an host alternating aphid *Aphis fabae* it is seen that the presumptive gynoparae when transferred to long day under crowded conditions a high proportion of the resulting adults develops as alatae, which shows the overriding effect of crowding on the long day apterising effect on gynoparae.(Hardie,1980b) Similarly, if Juvenile hormone-I treated presumptive gynoparae were crowded, only alate adults developed and under less severely crowded condition, 45% becomes apterised.

The photoperiodic receptor responsible for the photoperiodic control of morph determination in aphids, have been found to lie on the region of the protocerebrum (Lees, 1964). Histological studies have revealed the presence of a prominent group of around 8-12 neurosecretary cells arranged in two clusters on either side of the protocerebral cleft in the pars-intercerebralis.

These cells were considered homologous to the median neurosecretory cells in other groups of insects (Johnson, 1963; Von Gabriel, 1965;Steel, 1977).

The neurosecretary cells of aphids are distributed on the brain, sub-oesophageal and the abdominal ganglia. Johnson (1962; 1963) on the basis of his study of neurosecretion in several species of aphids, found that the neurosecretory cells in the brain of aphids carry neurosecretory material over to long distances through axonal transport.

Steel (1977,78), and Steel and Lees (1977) identified the neurosecretory cells in the brain and studied their role in the photoperiodic regulation of polymorphism in the aphid *Megoura viciae*, by employing cytological techniques and by selective ablation method employing radio frequency cauterisation.

Among these groups of cells, the Group-I neurosecretory cells have only been found responsible for the photoperiodic control of virginopara production under long day photoperiod. (Steel and Lees, 1977).

The role of these neurosecretory cells in the brain in relation to photoperiodism has been studied in the aphid *Megoura*, the axonal reservoir of these cells shows a presence of a large quantity of neurosecretory material. When these cells in individuals under long day conditions were ablated it was observed that the individual immediately revert to production of the sexual forms, further individuals that were producing oviparae failed to respond to a change over to long day photoperiod. Thus, it was suggested that a factor called 'virginoparin' is produced that acts on the ovaries and promotes then to develop into virginoparous forms. Whereas, there absence during shortday causes the production of sexual forms. (Steel, 1976;Mittler et al, 1979;Lees, 1980).

Direct evidences on the involvement of juvenile hormone secreted by the corpora allata in the regulation of morph differentiation is difficult to obtain because of the delicacy and size of these insects. Classical techniques employed in the study of insect endocrinology like allatectomy, parabiosis, and implantation cannot be performed. This was circumvented by the discovery of the antiallatin Precocenes from the plant *Ageratum houstonianum* (Bowers et al, 1978). Extracts from this plant was found to contain two active compound namely Precocene-I and Precocene-II. Application of this compounds to many hemimetabolous species of insects were found to induce a variety of physiological and behavioural changes which includes precocious metamorphosis of the immature stages, sterilisation of adult females, induction of diapause and inhibition of sex pheromone production. Precocene was thus found to aid as a powerful probe to investigate the hormonal regulation of metamorphosis, diapause, and reproduction as well as behavioural and further also in the study of morph determination involving the juvenile hormone.

The process by which Precocene exert this action has been partly understood. In Milkweed bug *Oncopeltus* it was found that Precocene inhibited the corpora allata development, and caused a diminution of the corpora allata volume, studies have revealed a direct involvement of Precocene on the corpora allata (Masner et al, 1979). It was shown histologically that Precocene treated Corpora allata (CA) cells get destroyed (Unnithan et al, 1977; Liechty and Sedlak, 1978) In *Scistocerca gregaria* it was shown that CA cells are completely destroyed and the gland is invaded by connective tissue. Studies have shown that an oxidative metabolite - probably an epoxide is formed by the action of the monooxygenase enzyme, which is involved in the synthesis of Juvenile Hormone.

Number of works have been conducted in aphids, using Precocene as a chemical probe to study the effect of juvenile hormone in morph determination and thereby to understand the role played by the Corpora allata. Mackauer et al (1979) found that topical application of Precocene-II to adult *Acrythosiphum pisum* leads to the production of alate progeny. In

Macrosiphum euphorbiae, Precocene II has been found to stimulate the production of alate (Delisle and Cloutier, 1980). Hales and Mittler (1983b) found that treatment of Precocene to adult female leads to production of males after a sequence of parthenogenetic progeny, this was the first report on the effect of Precocene in sex determination. In *Myzus persicae* Precocene treatment has been found to cause precocious adults, but no alate progeny was found to be induced (Hales and Mittler, 1981).

Precocene because of its anti-allatin activity is considered a potential fourth generation pesticide. However, the fact that it does not show response on the pleurometabolous insects can be of deterrent in its use as a general pesticide against all pest insects. But, never the less their effect on the hemimetabolous insect at least is well founded (Bowers, 1985). Being used mainly in the experimental study of the role of juvenile hormone in development and reproduction as a tool and its potential as an insect control agent has not been fully evaluated.

A number of attempts have also been made to utilise the insect pheromones in the control of insects. Pheromones causes a number of physiological as well as behavioural effects in the insects like aggregation, sexual attraction during mating, alarm etc. However most of these compounds have been found to be species specific.

One of such pheromones, the 'Queen substance' (9-oxodec-trans-2-enoic acid) is secreted by the queen honeybee (Butler et al, 1961). It inhibits the development of the ovaries in the worker female and inhibits the production of further queens. Apart from these, 'Queen substance' has been reported to cause similar effect on other groups of social insects like the ant *Formica fusca* L, (Carlisle and Butler, 1956) and the termite *Kalotermes flavicollis* F. (Hrdy et al., 1960) and in the house fly *Musca domestica* L. (Nayar, 1963). 'Queen substance' has been found to be innocuous to the immature stages of the mosquito *Aedes aegypti* L, except during the larval and pupal moult. A delayed toxic effect was observed; treated insects sickened and died during the pupal stage (Sayeed Qurashi and Thorsteinson, 1965).

Therefore, an attempt is made to study the morphological as well as physiological effects of Precocene and 'Queen substance' as a control agent in aphids, so as to gain an understanding on the possibility of its use as an pesticide by its growth disruptive activity could yield valuable information.

In a number of aphids, Precocene application has been found to cause a number of effects on the developing as well as their progeny born. In *Aphis craccivora* (Koch) Precocene-II treatment leads to reduction in the progeny numbers as well as in the reduction of life span of the adult.

Precocious metamorphosis is also observed in the developing nymphs (Srivastava and Jaiswal, 1989).

Thus, the present study aims at understanding the phenomenon of polymorphism in the aphid *Macrosiphum rosaeiformis* (Das), and to gain an insight on some environmental and endocrine factors responsible for the differentiation of various morphs. Further, to investigate the potential of compounds like Precocene and 'Queen Substance' as a potential agent for the control of aphids.

LIFE CYCLE AND POLYMORPH DETERMINATION

Plate-1: Photograph showing a cluster of *Macrosiphum rosaeiformis* (Das) feeding on Rose plant.

INTRODUCTION:

Macrosiphum rosaeiformis is a common pest infesting all varieties of garden as well as cultivated roses (*Rosa* sp.). It attacks the tender flower buds, young growing twigs and leaves. Heavy infestation of these insects causes deterioration in the quality of flowers. It is thus an important floricultural pest of economic importance, as rose constitute a major profit-generating sector in the floriculture industry. Further, a number of oils are also extracted from rose for the perfume industry.

Macrosiphum rosaeiformis is cosmopolitan in its distribution and are found to occur through out the year. Taxonomic description of this species is given by a number of workers (David, 1975;Roychudhury, 1980) Literature on other aspects of its biology are sparse. Some aspects of biology of this species have also been studied (Atwal and Dingra, 1971; Rana and Bisht, 1989), bioecological studies and control of this aphid have also been done by Kaker and Sood, (1989). Considering the vast geo-climatic variation within the states of India, it is understandable that life cycle exhibited by this insect might show some interesting variation, since it is known that environmental factors like photoperiodism and temperature influences the life cycle and biology of an insects (Dixon, 1985;Mackay, 1987). Therefore the present observations aims at determining the occurrence of the various polymorphic forms under the environmental factors mainly photoperiod and temperature conditions and thus to obtain a picture of its life cycle.

M.rosaeiformis (Das) is believed to be an anholocyclic and monoecious species, migration during the winter months is observed to take place to the warmer plains to escape the rigors of the approaching winter and depleting food sources (David, 1975). A few polymorphic forms have been mentioned in this species. These include the alate and the apterous virginopara, the egg laying sexual ovipara and the males. There is however no mention of any gynoparous forms, neither of any intermorphs forms have been reported so far. Although, the presence of these parthenogenetic as well as Gamic morphs suggested a possible Holocyclic mode of life cycle, but exact details of its life cycle is however not available so far.

Aphids in general exhibit three types of life cycle. (a): Anholocyclic, autoecious, (b): Holocyclic, autoecious and (c): Holocyclic, heterogeneous. Holocyclic may be either complete or incomplete, in which case life cycle is completed entirely on a single host (autoecious) unlike in the case of the heteroecious forms where life cycle alternates between the primary and the secondary host plant.For example, in case of the aphid, *Aphis fabae,*which is holocyclic, heteroecious, the parthenogenetic virginoparous form give rise to the gynopara under a condition of reduced photoperiod .These gynoparous forms further leads to the production of the egg laying Ovipara.These parthenogenetic and the gamic forms alternate between two host plants during summer and winter periods. In case of holocyclic, autoecious types such as in *Megoura viciae,* ovipara are produced directly by the parthenogenetic virginopara. In anholocyclic,autoecious types like *Myzus persicae*, life cycle is entirely through means of parthenogenesis and the alate form migrates within a single species of host plant, and there is thus, no annual cycle of host alteration. (Hardie and Lees, 1985).

In case of host alternating species there may be up to five or more distinct morph forms, which includes the asexual viviparous alate and apterous females, the sexual egg laying ovipara, the males, and the fundatrix. The sexual forms are either alate or apterous. In several groups of aphids, one or more of these morphs may themselves be polymorphic, so that three different kinds of viviparous alates and the same number of apterous vivipara may occur during the course of its complete life cycle (Hille Ris Lambers, 1966).

Due to the complexity of the life cycle and the number of polymorphic forms, aphid biologist use specific terminology for description of each morph, A brief nomenclature of the various morphs of aphids is mentioned below:-

Alate : winged forms

Apterous : wingless forms.

Virginopara : Female individual which reproduces solely through means of parthenogenetic viviparity.They may be either alate or apterous.

Vivipara: Female morphs that give birth to live young progeny.

Gynopara : alate morph producing exclusively the egg laying ovipara,they are usually induced by short photoperiod.

Mixed Gynopara: Alate morph producing other morphs as well as oviparae.

Ovipara : the sexual female that lays the overwintering eggs.

Presumptive alate virginopara: A crowded first instar larvae born to a long day apterous virginopara which if reared in long days would revert to alate virginopara.

Presumptive gynopara: A first instar larva which has received prenatal short days and which if reared postnatally in short days would become a gynopara.

There is a division of labour between the more or less sedentary juvenile individuals, which are involved mainly in feeding and growth, and on the other hand the active, reproductively specialised adult. Further, the division of labour among the alternative forms of aphids is of the same kind. In the apterous vivipara, the juvenile assimilatory functions are most highly developed and the sensorimotor functions are reduced in the adult as well as in the larvae of the apterous vivipara. On the other hand the non-assimilatory, sensorimotor functions of food seeking, mating and oviposition are elaborated in the winged sexual forms (Hille Ris Lambers, 1966).

The parthenogenetic form of reproduction prevails during the warm season with the availability of abundant food source as well as favourable environmental conditions.This leads to a high rate of progeny production and a rapid proliferation that leads to emergence of alate forms which migrate to newer food sources . But during the approaching winter months, with its inclement weather conditions, the sexual forms arise and thus ensures the survival of the species by producing the diapause eggs that overwinter in the primary or generally the secondary host plants depending upon life cycle and gives rise to the fundatrices in the spring season (Lees, 1966;Hille Ris Lambers,1966). Life cycle thus alternates between the parthenogenetic viviparous and the sexual oviparous forms.

The phenomenon of polymorphism is thus a biological strategy of its life cycle that enables the aphids to survive the changing physical conditions of their environment. An alteration between the asexual and efficient parthenogenetic reproduction during a bad season and the sexual mode during approaching mean season guarantees that the survivability of the species in case of any environmental contingencies is ensured. (Ghosh and Roychoudhury, 1986-87).

The factors' governing polymorphism is mainly environmental and is mediated via the endocrine system. Conditions like Photoperiodism, temperature, crowding, nutrition and plant quality are the main stimuli that acts on the developing individuals and determine the course of their specific pathway of development leading to a specific morph (Hille Ris Lambers, 1966: Lees, 1966).

The chief environmental factor that controls morph differentiation as well as the mode of life cycle is Photoperiod coupled with temperature. Marcovitch (1924) observed for the first time the connection between daylength and the seasonal appearance of sexual forms in the aphid *Aphis forbesi.* Such responses have been found also in case of other species of aphids,for instance in *Brevicoryne brassicae,* (Bonnemaison,1951), *Acrythosiphum pisum* (Kenten,1955; Lamb and Pointing, 1972; Sharma et al, 1973;MacKay et al, 1983; MacKay,1987), *Megoura viciae* (Lees, 1959,60,63). (Bonnemaison,1951),

Another, factor responsible for the determination of the alate morph is through crowding.Crowding brought through by overpopulation of available feeding site, which acts either prenatally or post-natally on the developing embryos or early instars and switchover takes place towards alate development depending upon species. The effects of crowding has been observed in a number of species of aphids which includes *Megoura viciae,* (Lees, 1961; Lees, 1966), *Muzus persicae* Sulzr, (Sutherland and Mittler, 1971), *Aphis craccivora* (Johnson, 1965).The actual mechanism by which crowding induces the production of alates is however not known (Lees, 1978;Hardie and Lees, 1985).

MATERIALS AND METHODS:

Aphids for this study were reared in potted rose plant (*Rosa sp.*) kept inside a screened net house under outdoor conditions. Monthly observation were made by collecting the individuals.These were killed and processed and mounted in slides for microscopic examination of various characters and morphs. For the study on the effect of photoperiod and temperature on morph production, aphids were collected from screened house and were subjected to specific photoperiod and temperature in a BOD incubator.The photoperiod consisted of a photophase of 6L:18D, 8L:16D,12L:12D and 16L:8D (Light:Dark). For the sequence of morph production, parent individuals were subjected to a photoperiod of 8L: 16D at a constant temperature of 10°C±2°C and the progeny maintained till adult. The percentage of their morphs was determined. Insects were kept on 6-inch diameter petridishes and feed on excised fresh leaves (*Rosa* sp), the leaves were kept fresh by soaking the petioles with moistened paper towelling soaked with water. (MacKay and

Downer, 1979; MacKay et al, 1983). Relative humidity inside the BOD was kept at around 60% - 80%.

Observations were made at regular intervals, the progeny born were allowed to continue till adult, and their morphs determined for each batch of experiments.

OBSERVATIONS:

During the course of the present study the following morph forms were observed (Table 2).The terminology used for their description are as per that of Hille Ris Lambers, 1966.

Virginopara : They are the parthenogenetically reproducing females. These are either alate or apterous. The alates are characterised by the presence of paired wings, the pterothorax which is highly sclerotised and dark in colour. Secondary rhinaria count on the III antennal segment of the alates are numerous, numbering about 41.27 ± 3.98 (SD). They are distributed on about 90% of the basal half of the III antennal segment. The marginaL sclerite on the abdominal segments are present. The alates are distinguished only during their early third instar when the wing buds appear as small swelling on either side of the mesothorax.

Apterous virginopara are comparatively larger in their size as compared to the alates, the thoracic region is unsclerotised, marginal sclerite on tergites are faint; secondary rhinaria on III antennal segment are few and are distributed on basal 25% of the segment, and numbering about 13.41 ± 2.57(SD).

Ovipara: Either winged or wingless, their chief characteristic are the presence of scent plaques on the hind tibiae numbering about 70.83± 25.48 (SD) in the alate forms, third antennal segment bearing around 33.6 ± 5.67 (SD) secondary rhinaria, superficially they resemble the alate vivipara except that the abdominal pigmentation is light in colour and comparatively smaller than the virginopara.

The apterous forms are smaller with swollen hind tibiae which is entirely dark in colour bearing numerous scent plaques,around 201.0 ± 1.41 (SD), and is comparatively more than that of the alate forms (around 70.83). The secondary rhinaria on the third antennal segment is relatively less in numbers. Apart from these, the scent plaques are found to be larger in case of the apterous forms.

Gynopara : They are morphologically similar to the alate virginopara, but differ significantly in their reproductive physiology. They arise under the conditions of reduced photoperiod and temperature and give rise to the egg laying Ovipara. The antennal sensillae on the III segment number about 57.5 ± 3.96 (SD), which is comparatively more than the alate virginopara which has about 41.27 ± 3.98(SD). This morph is observed only during the winter months. They can be easily confused with the alate virginopara, unless they are brought to the laboratory and reared to see the progeny morph which are mainly oviparae together with some percentage of virginoparae.

Apart from these usual morphs, it was observed that some morph forms exhibiting characteristics intermediate between two morphs are also observed mainly during the approaching and receding winter months. Some of the intermediate morphs observed are:

Intermediate Ovi-vivipara: They are characterised by the presence of both oviparous and the viviparous features. The hind tibiae have scent plaques that number about 140 approximately, and the abdomen contains embryos instead of haploid eggs. Genital plate present or ill developed and they are non-reproductive. They are found to occur during the period prior to the appearance of the oviparous forms. (Plate 2)

Intermediate alate - apterous: They are individuals possessing characters with varying grades like reduced and crumpled wings, unsclerotised pterothorax, some with only small wing buds and having apterous features.

They are reproductively normal and are found to occur mainly at the beginning of the approaching winter months starting November and found to occur till late April; however they are absent totally during the intervening period. They are likely to be the gynoparous forms.

Effects of Photoperiod on alate production:

The effect of photoperiod on alary morph production was studied on the IV instar apterous virginoparae. When these individuals were treated at different photoperiods , at a temperature of 10°C ± 2°C, it is observed that the progeny produced by the individuals treated at a Photoperiod of 8L: 16D produced maximum alates. An increase in the photoperiod to 12L: 12D leads to a lowering of the alate morphs in the progeny and under 16L: 8D no alate progeny were observed. A decreased in the alate progeny was also observed under a photoperiod of 6L: 18D. (Fig. I)

Production of Gynopara and Ovipara under reduced photoperiod and temperature:

Experiment was also conducted to see the role of short photoperiod and low temperature on the induction of sexual morphs. It was found that when apterous virginoparous individuals were subjected to short photoperiod consisting of a photophase of LD 8:16, and temperature 10°C ± 2°C, the progeny born were mainly alate (Fig. 2 & 3). These alates when continued further give birth to the egg laying ovipara. Thus, ovipara are produced by the short day induced alate vivipara, or the Gynopara. These alate vivipara produced by short day treatment are similar to the long day vivipara morphologically but differ only in their ability to produce the sexual female or the ovipara. The secondary rhinaria count on the third antennal segment in the gynopara is however more than that of the alate vivipara produced under long day conditions.

Both the alate and the apterous virginoparous individuals on treatment show the sequence of morph production under short photoperiod and low temp. In case of the alate individuals treated, the first generation progeny are mainly apterous, whereas, in case of the apterous percentage of alate is maximum in the first generation progeny.

Alate male individuals were observed under natural conditions.They are smaller than the vivipara and are characterised by their small abdomen, presence of genitalia as well in having a darker body coloration. The antennal sensillae are distributed an all the third, fourth, fifth and the sixth segments. (Plate 2)

Table –1 Showing the number of Secondary rhinaria and scent plaques in the antennal and hind tibiae respectively of various morphs.

Morph Type	I Antennal segment			Hind tibiae			
	Mean	±	SD	Mean	±	SD	(n)
Apterous Virginopara	13.41	±	2.57	0		0	(25)
Alate Virginopara	41.27	±	3.98	0		0	(24)
Alate Gynopara	57.5	±	3.96	0		0	(7)
Alate Ovipara	33.6	±	5.67	70.83	±	25.48	(6)
Apterous Ovipara	09.25	±	0.5	201.0	±	1.41	(2)
Alate Ovi/viviparous Intermediate	38.5	±	5.72	140.0	±	0.00	(2)
Alate/apterous Intermediate	39.75	±	0.35	0.0		0.0	(2)

Table-2

Month wise occurrence of various morphs based on observation during the year 1995-1996.

Morphs	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Apterous Virginopara	+	+	+	+	+	+	+	+	+	+	+	+
Alate Virginopara	+	+	+	+	+	+	+	+	+	+	+	+
Gynopara (Alate)	+										+	+
Ovipara (Alate)	+	+										+
Alt/Apt Intermediate			+	+						+	+	+
Ovi/Vir Intermediate	+	+									+	+

+ Present

Plate-2: Plate showing the distribution of secondary rhinaria and scent plaques in the antennal segment and hind tibiae

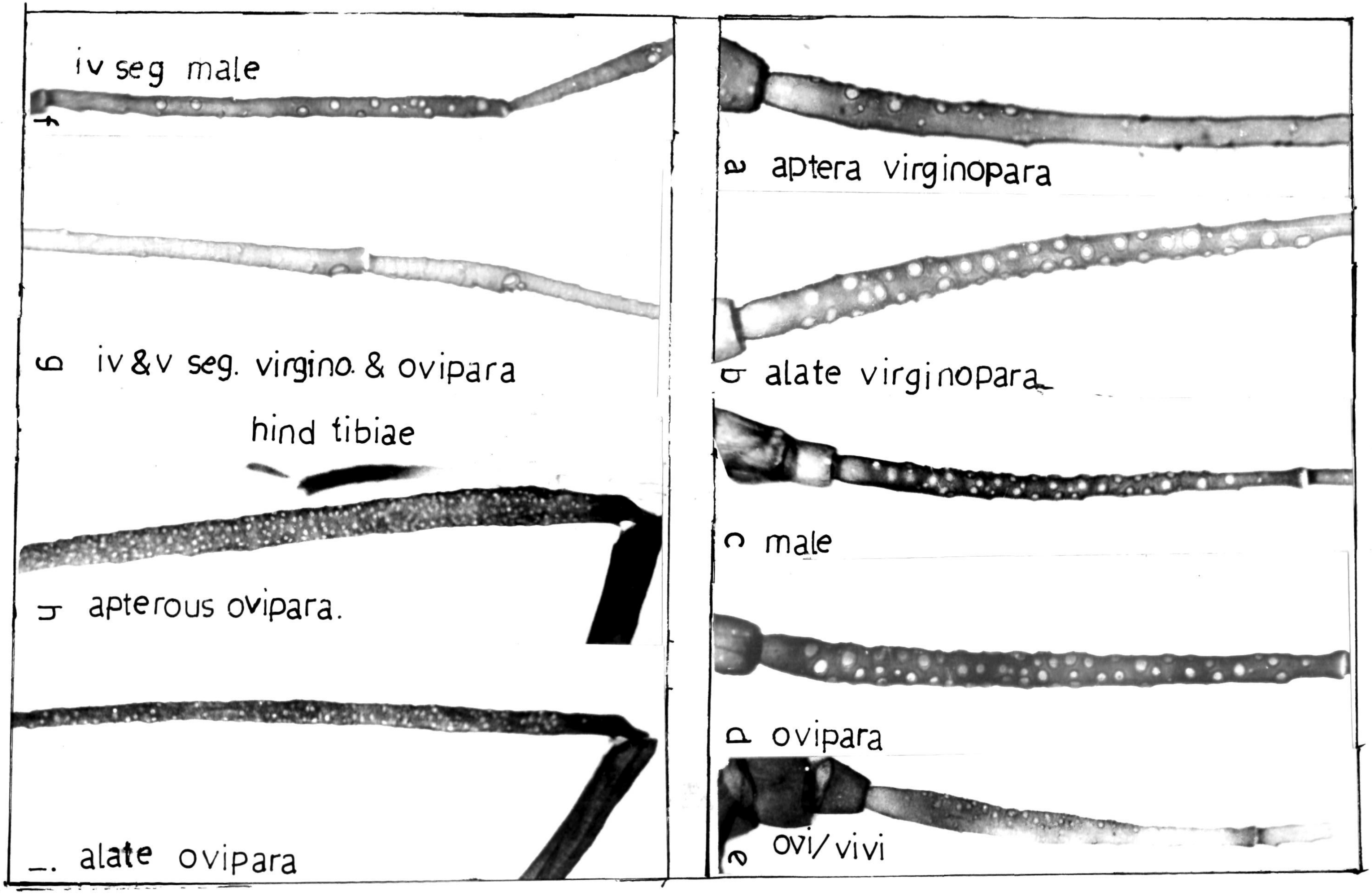

a aptera virginopara
b alate virginopara
c male
d ovipara
e ovi/vivi
f iv seg male
g iv & v seg. virgino. & ovipara
hind tibiae
h apterous ovipara.
i alate ovipara

Plate-**3**: Photograph showing the oviparous/ virginoparous intermediate form.
Em- embryo, ht- hind tibiae, C-cornicle. Ge- genital plate.

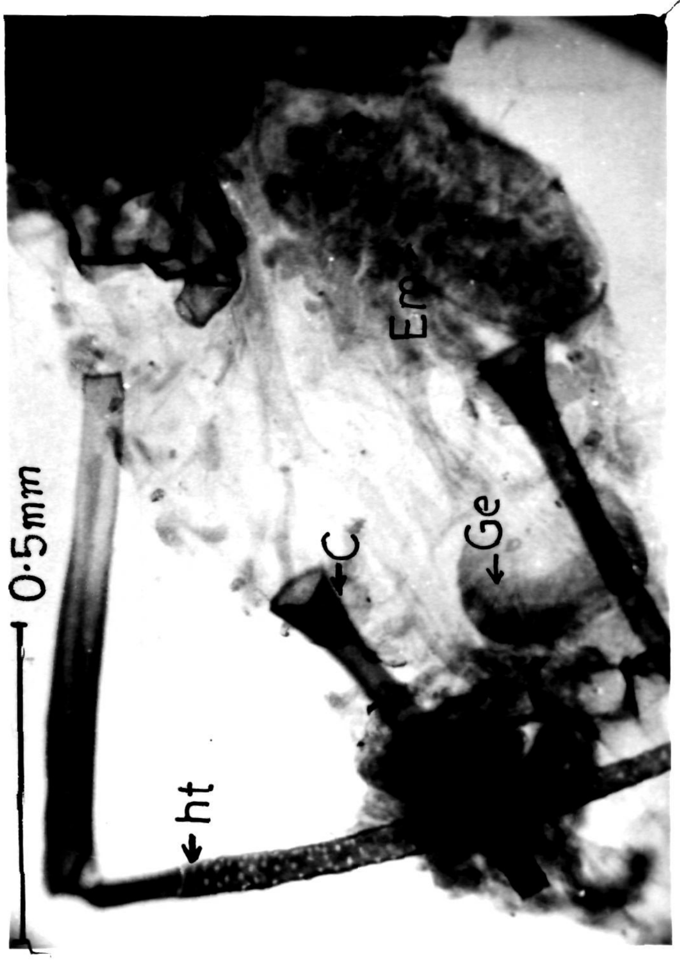
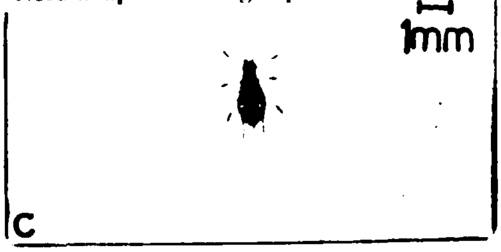

0·5mm
Em
C
Ge
ht

Plate-4: Plate showing normal and alate/apterous intermediate form.

Alate/ apterous virginopara

Normal alate Virginopara



Normal apterous Virginopara

Fig-1: Histogram showing the production of alate under short photoperiod.

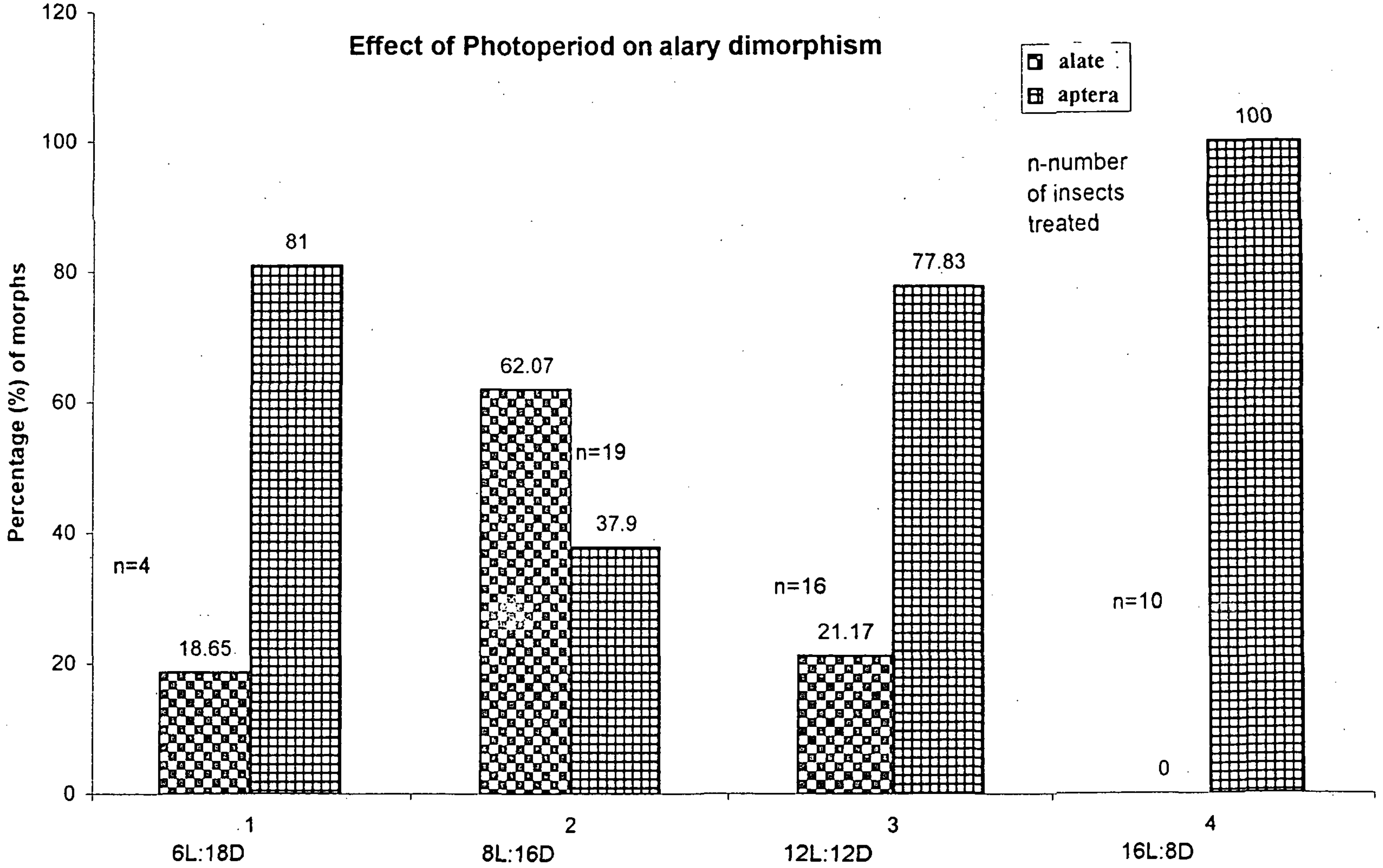

Effect of Photoperiod on alary dimorphism
alate
aptera
n-number of insects treated
Percentage (%) of morphs
120
100
80
60
40
20
0
n=4
18.65
81
62.07
n=19
37.9
n=16
21.17
77.83
n=10
0
100
1
6L:18D
2
8L:16D
3
12L:12D
4
16L:8D

Fig.1

Fig-2: Histogram showing the sequence of morph production under reduced photoperiod by apterous virginoparae.
G1-generation 1, G2-generation 2, G3- generation 3
Number of parent individual treated = 15

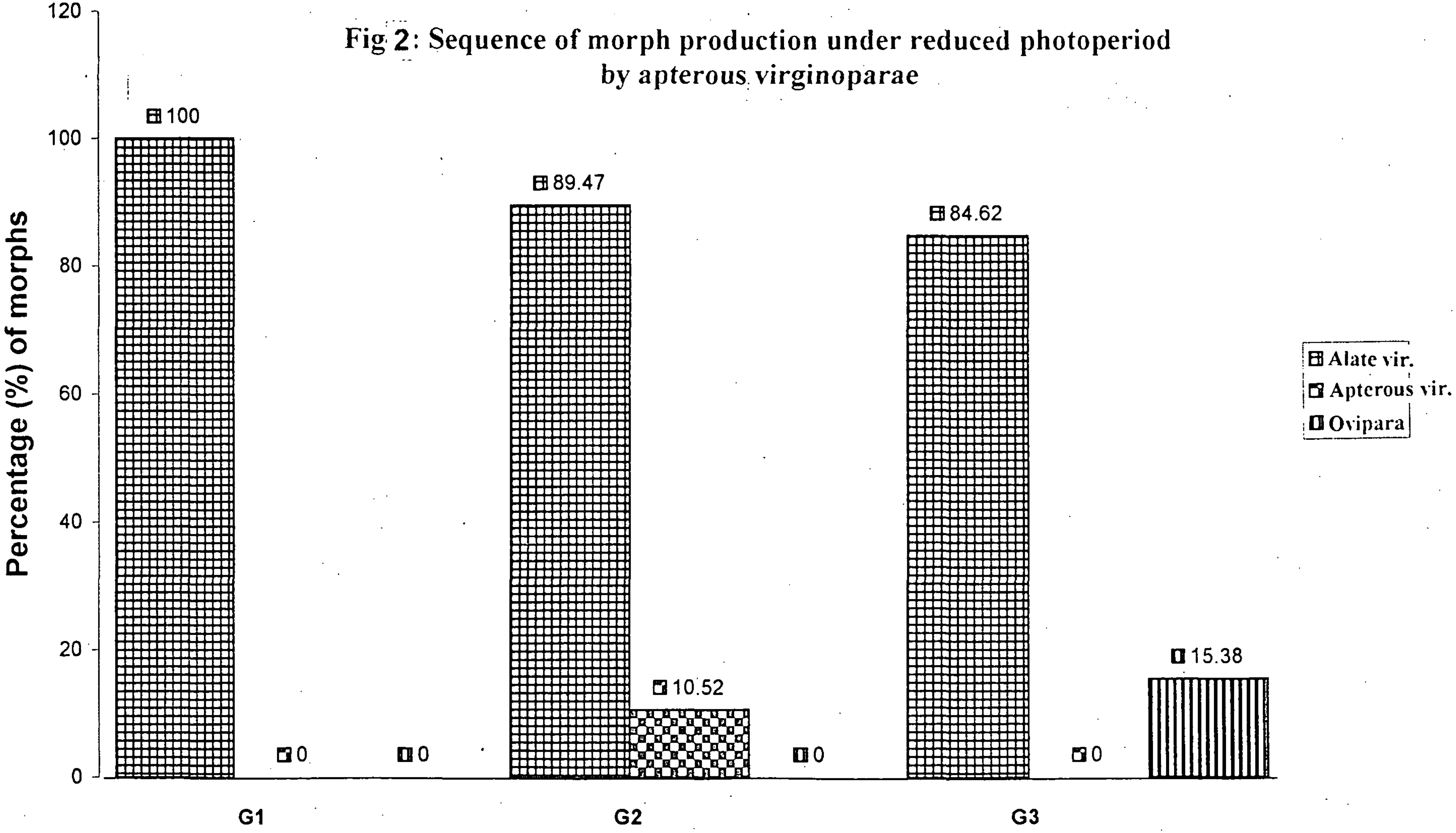
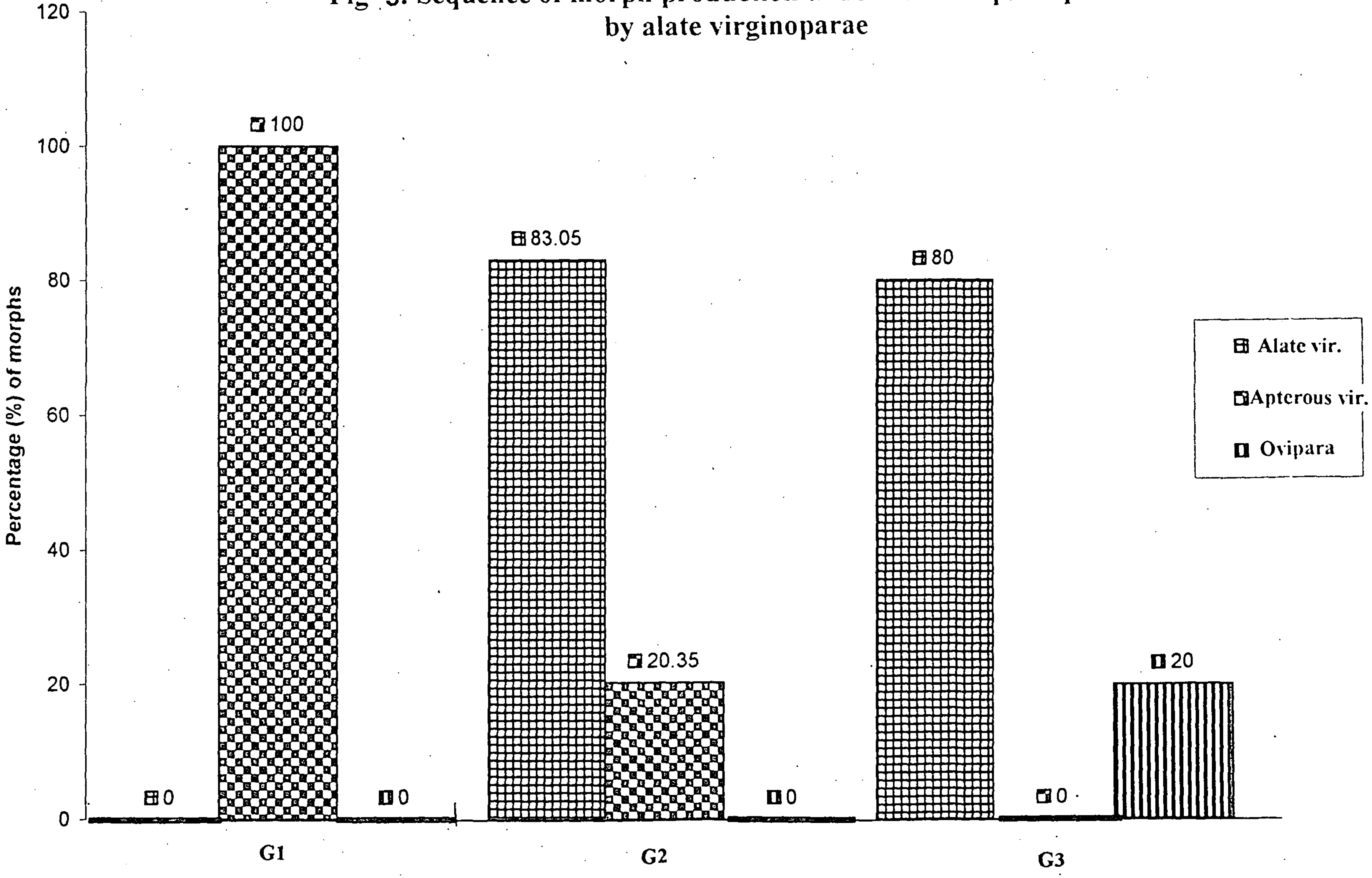


Fig 2: Sequence of morph production under reduced photoperiod by apterous virginoparae

Fig-3: Histogram showing the sequence of morph production under reduced photoperiod by alate virginoparae.
G1-generation 1, G2-generation 2, G3- generation 3
Number of parent individual treated = 15

Fig- 3: Sequence of morph production under reduced photoperiod by alate virginoparae



DISCUSSIONS:

The presence of these morphs in *Macrosiphum rosaeiformis,* thus indicates the presence of both the parthenogenetic as well as the gamic forms, along with the many morphs associated with it. Each polymorph form is distinct and is suited for the demands required during the changing environmental factors like food source, space, photoperiodism and temperature. Thus, there is an apparent division of labour that enables them to survive in wide range of environmental conditions and host plants. (Kennedy and Stroyan, 1959; Dixon, 1985). The parthenogenetic virginoparous forms are prevalent mainly during warmer period of the year, their feeding site are the tender flower buds and shoots, a high breeding rate results in crowding of the available feeding site, tactile stimulation due to crowding thus induces the production of alate forms which fly to new food source.

In many species of aphids a short photoperiod have been found to significantly increase the production of alate virginoparae (Johnson, 1966a). In case of host alternating species of aphids the alate produced under short photoperiod are actually the gynopara which produce the sexual form, i.e the ovipara. (Bonnemaison, 1951; Blackman, 1975; Dixon, 1971). Thus, the alate produced by *Macrosiphum rosaeiformis* under the influence of short photoperiod are essentially the short day gynoparae.

The effect of crowding also influence alate production as has been mentioned already, Apart from the influence of crowding alate forms have been found to arise due to factors like deterioration of host plant and thereby lowered nutritional quality. In the aphid *Chaetosiphum fragaefolii*, more alate are produced by parent, when reared in old leaves than in fresh leaves. (Schaefers and Judge, 1971). Thus , it is possible that the alate viviparous forms observed under summer long day conditions produced as a result of these factors.

The alates produced under long photoperiod under the influence of crowding etc. are specialised virginoparous forms, they not only differ in the presence of wings, but also on the distribution of the antennal sensillae, which is relatively more than in case of the apterous form. Apart from this they are smaller and have a strong urge to fly, they probably differ from the apterous virginopara in their reproductive physiology and strategy also (Dixon, 1985). The short day alate virginopara or the Gynopara on the other hand born during the winter months are although morphologically similar with the long day produced virginopara, but differs on the number of secondary rhinaria. Secondary rhinaria is found on the third and the fourth segment of the antennae and differences has been found in other species of aphid as well. In *Myzus persicae* the secondary rhinaria in the third antennal segment differs between the alate virginoparae and the gynoparae. (Takaoka, 1960), in *Rhapalosiphum padi*, segment third and fourth and in *Rhaphalosiphum crataegellum* in the 4th segment (Rogerson, 1947). In case of the aphid *Aphis fabae* differences have been found on the distribution on the 3th and 4th segment (Jones, 1944; Kennedy and Booth, 1954, Hardie, 1980).

The appearance of sexual female and males during winter indicated the role of shortening photoperiod and temperature in its determination. The influence of short photoperiod and low temperature on the virginoparous forms act in a way whereby the progeny born immediately are the alate virginopara, which on further subjection to short day gives rise to the ovipara. Thus, the effect of photoperiod act on the parent generation and sequential batch of distinct morph forms are produced in the succeeding generation. This is explained as being due to the maternal effect being mediated by the endocrine system (Lees, 1966; Mousseau and Dingle, 1991)

Although we observed the occurrence of males in the outdoor conditions, no males were noticed under laboratory conditions. This is most likely due to the sequence in which the progeny morphs are produced. In most aphids, males are born latter during the progeny sequence, for example in the aphid *Acrythosiphum pisum* males are born the last, after about fifty females (Kenten, 1955).

The production of intermediate forms has also been documented in other species of aphids (Lees, 1966; Hardie, 1980). Tsitsipis and Mittler (1976a, 1977a) described apterous morphs resembling either oviparae or virginoparae, with ovaries containing haploid eggs and embryos. Although, such intermediate forms have been reported, but no previous information on the occurrence of intermediate morphs of aphids in this part of India is available. (Roychoudhury, 1980). The occurrence of these intermediate morphs in the natural environment is of significance in understanding the physiological processes of aphid polymorphism as well as of importance to aphid systematics. It is established that environmental factors mainly photoperiod and temperature in conjunction with the endocrine system plays a significant role on the determination of polymorphic forms. Studies in *Megoura viciae* has shown that the median neurosecretory cells as well as Juvenile hormone from the corpora allata are responsible for the photoperiodic determination of the viviparous form under long-day condition (Steel and Lees, 1977). Oviparous-viviparous intermediate forms are known to occur at critical photoperiod or temperature and also at the switch-over points in the progeny sequence when the progeny maternal photoperiod is reversed (Lees, 1966), thus, the apparent factor or influence is not maintained during the entire sensitive period of development of the presumptive embryos.

In case of the alate-apterous intermorph the mechanism is possibly same except that these intermorphs are infact the partially developed alate

Fig-4: Schematic representation of the life cycle of *Macrosiphum rosaeiformis* (Das).

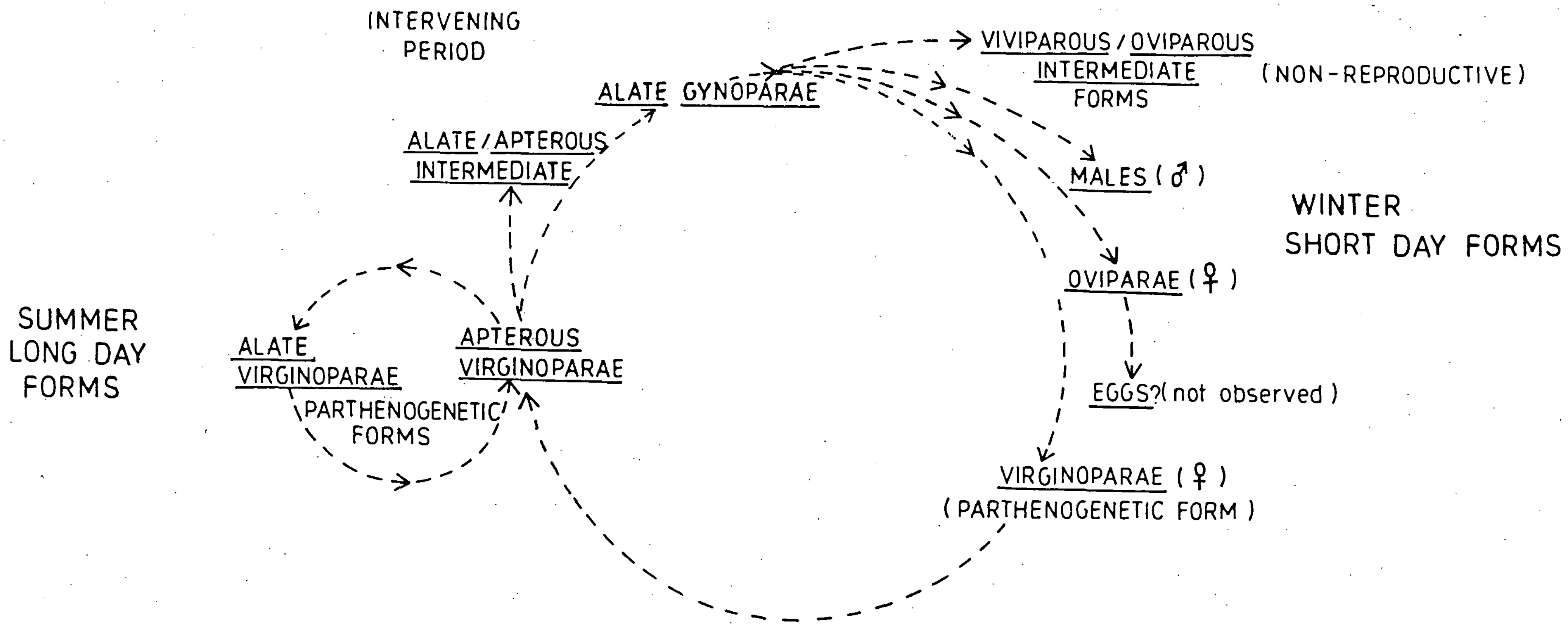


Fig-4

SCHEMATIC REPRESENTATION OF THE LIFE CYCLE OF THE APHID, _MACROSIPHUM ROSAEIFORMIS_ (DAS).

gynopara which fails to express fully, this forms are mainly observed for a very short interval; prior to the approaching winter months.

Thus, from the present observation it is seen that *Macrosiphum rosaeiformis* is a highly polymorphic species and the life cycle appears to be Holocyclic but incomplete (Fig 4), since no sexual reproduction has been observed and no eggs were found during the course of the present observations.

JUVENILE HORMONE AND MORPH DIFFERENTIATION

INTRODUCTION :

The endocrinological events involved in the phenomenon of alary dimorphism in aphids is a process very little understood (Lees, 1966,1983;Hardie and Lees, 1985; Nijhout and Wheeler, 1982; Nijhout, 1994). It is generally believed that juvenile hormone secreted by the corpora allata, apart from its role during the process of metamorphosis is also involved in the determination of alate and the apterous forms (Wiglesworth, 1955; Lees,1961) The apterous form because of its morphological resemblance to the young instars stage was thus considered to be a neotenous form, being brought about by a supposed enhanced level of juvenile hormone.

With this notion, early workers attempted to reverse the alate condition by topical application of juvenile hormone extract from the abdomen of adult *Cecropia* moth, as well as with JH mimics . In *Megoura* application of this hormone extract exhibited striking results especially on the treated third and fourth instar alatae (Lees, 1961,1966). The treated individuals undergoes normal moulting and become reproductive. The genital duct, genital plate, cauda and the appendages remained that of the normal adult type, further the ocelli and the plate organs also remained unaffected, but the site of hormone application, the mesonotum remained often completely unsclerotised, general pigmentation of the mesothorax also become lighter and the sutures between neighbouring sclerites tend to become obliterated. Further, the wings becomes crumpled and reduced in size. The abdominal marginal sclerite also disappears entirely.

Application on the first and second instars also failed to produce a form reversal (Lees, 1966). Further, the application of juvenoid with higher JH activity showed that middle instar alate virginoparae, when treated topically under long day conditions formed into supernumerary larvae in the IV instar, instead of an adult. This V instar subsequently undergoes further one more ecdysis (Lees, 1966: 1980; White, 1968; White and Gregory, 1972). The fourth instar larvae so formed possessed alate larval features. White (1971) noted a partial suppression of wing development in *Brevicoryne brassicae* when its third instar larvae were treated with JH analogue.

The effects of Juvenile hormone prenatally on the progeny were also studied. It is found that, application of juvenile hormone and its mimics in most cases leads to an increase in the apterous morphs in the progeny. The apterous forms produced after juvenile hormone application on *Aphis nerii* and *Aphis craccivora* shows absence of secondary characters associated with alate forms, this includes the antennal sensoria and chitinised patches under the cornicles (Srivastava and Chhibber,1980). It is seen that in case of the aphid *Aphis fabae* treatment of the second instar larvae with JH analogue have been reported to lead to a decrease in the proportion of alates (Von Dehn,1963). In *B.brassicae*, when synthetic JH is applied to both parents and progeny an increase in the numbers of apterous progeny were observed in case of prenatal application with higher dose, post-natal application to first instar larvae also appeared to reduce the numbers developing into aptera at a lower dose, treatment of second instar were without any effect (White and Lamb, 1968; White, 1968a). Further a partial suppression of wing development was noted when the 3rd instar larvae were administered juvenile hormone analogue (White, 1971).

Administration of chlorinated farnesoic acid and synthetic JH as well as ecdysterone topically in *Myzus persicae* were found to be without any effect (Applebaum et al, 1975), but synthetic juvenile hormone administered with artificial diet were reported to induce apterous development (Kohno and Takaoka, 1977). In case of the aphid *A. nerii* application of juvenoid or juvenile hormone analogue have been reported to cause apterisation of progeny both pre- and post- natally (Srivastava and Chhibber, 1980).

In *Brevicoryne brassicae* treatment with juvenile hormone analogue to the 3rd instar larvae leads to partial suppression of wing development, treatment of 4th instar or adult shows an increased production of apterous in the progeny. Further, it was observed that treated 3rd and 4th instar brought about a reduction in wing size or caused abnormalities in wing structure in the developed adults, the progeny produced by such adults were cent percent apterous. (White and Lamb, 1968; White, 1968).

Based upon these observations it was deduced that treatment with juvenile hormone analogue causes the arrest of wing development at an early stage but once the wing flap have formed the treatment affects only the process of wing growth and differentiation

But criticism of these experiments have been made on the ground that the apterous and alate virginopara in these experiments were produced under conditions of long day and under isolation or crowding. This has the disadvantage because the proportion of alate is highly variable and often below 50% (Hille Ris Lambers, 1966). The developmental fate of an individual first instar larva therefore cannot be ascertained with certainty.

Photoperiodism plays a important role in the determination of alate morphs. Under the influence of long day photoperiod apterous virginopara in presence of stimulus such as crowding, nutrition, plant quality and interspecific interactions etc. gives birth mainly to the alate virginopara. The control mechanism in this case have been found to be operative either pre- or postnatally or both depending upon the species (Hardie and Lees, 1985). However, the alates virginopara produced under the influence of short day differ from the long day forms in their reproductive physiology in that; they produce a mixed progeny of gynoparous and oviparous forms. The factor regulating the alate gynopara production have been found to be as a result of reduced juvenile hormone level, it has been demonstrated in *A.fabae* that externous application of juvenile hormone to short day treated individuals have been reported to inhibit the percentage of alates progeny, further these SD presumptive alate gynopara can be revert back to apterous forms by subjecting them to long day photoperiod (Hardie ,1980).

However, the mechanism involved in the alate production under long day photoperiodic conditions is entirely different and is not clearly understood as in case of short day. It is observed that under long day the influence of population density act as the overriding factor .The effect of crowding however cannot be controlled and the percentage of alate progeny produced thus, cannot be predicted with certainty. (Hardie and Lees, 1985).

The problem is further enhanced by the size and delicacy of these insects. Techniques which was successfully applied in probing the role of the gland/hormone employing, namely allatectomy, parabiosis, implantation in the case of larger insects cannot be applied in aphids. However, with the discovery of Precocene compounds, extracted from plants by Bowers et al (1976), it has come to an aid in such cases. Precocene acted as a chemical agent destroying the corpora allata in many hemimetabolous insects. Thus, it helped immensely in understanding the role of corpora allata / juvenile hormone involved in the regulation of a number of phenomenon in insects. Precocene was thus considered as an effective tool in probing the phenomenon of aphid polymorphism.

Precocene treatment in most insects leads to the selective destruction of corpora allata, which involves the epoxidation via glandular enzyme required during juvenile hormone biosynthetic pathway, the precocene epoxide that forms acts as a general cytotoxic (Brooks and Mc Cafferery, 1990).

Experiments involving topical application of precocene compounds in a number of aphid species have however yielded unequivocal and contradictory results. In the aphid *Acrythosiphum pisum*, precocene-II (6,7-dimethoxy-2, 2-dimethyl chromene) applied prenatally induces production of alate progeny,but do not appear to lead to precocious metamorphosis (Mackauer, et al 1979;Hardie,1986). In *Macrosiphum euphorbiae* (Thomas) also precocene-II prenatally applied increases alate progeny, and also induces precocious adult development (Delisle et al,1983). In the Mustard aphid *Lipapis erysimi* (Kaltenback) precocene-II application on second instar larvae as well as to adult leads to an increased alate production (Rup and Sohal, 1989). In *Aphis fabae* and *Megoura* treatment with precocene I,II and III have been observed to induce alate progeny (Hardie,1986).In *Myzus persicae* also no increase in alate progeny is observed even after P-III application. However, P-III (6-methoxy, 7-ethoxy2, 2-dimethyl chromene) appears to inhibit wing development in presumptive winged aphids (Hardie, 1987;Hardie et al, 1995). It also provokes the development of precocious adult in the third or fourth stadium. Gao and Hardie (1996) found that Precocene-II applied to newly born *Acrythosiphum pisum* (Harris) does not influence development and observed that the pre-natal induction of wing progeny is as a result of maternal control mechanism.

cAMP (cyclic adenylate monophosphate) is refereed as second messenger and an increase in their intercellular concentrations triggers a cascade of biochemical reactions. In experimental endocrinology, it is often possible to mimic the action of a hormone on a particular target tissue by exposing that tissue to elevated concentration of exogenous cAMP. (Nijhout, 1994)

cAMP is known to mimic the action hormone in most insects, for example , it stimulates the secretary activity of malpigian tubules in many insects (Berridge,1970). cAMP have also been found to increase mating duration, reduce significantly the fecundity and fertility in *Dysdercus* sp. (Dutta and Banerjee, 1978).

The effects of cAMP on polymorphism in aphids is not known, it may be possible that the hormonal regulation of morph determination can be induced by a elevated level of this second messenger cAMP thereby acting directly at the level of the genes responsible for specific morph expression induced under specific environmental factors. Thus, an attempt is made in this study to observe the effect of cAMP, if any in the *Macrosiphum rosaeformis.*

MATERIALS AND METHODS:

Chemicals:-

Juvenile hormone:III-(Cis-10,11-Epoxy-3,7,11-trimethyl-trans ,trans -2,6-dodecadienoic acid methyl ester) was obtained from Sigma Aldrich USA. Required dose of the hormone was prepared in Spectroscopy grade acetone.

Precocene-I (7-methoxy-2,2-dimethyl chromene) and Precocene-II (6,7-dimethoxy-2, 2-dimethylchromene) was obtained from Sigma Aldrich,dissolved in required quantity in Acetone.

cAMP : Dibutryl cAMP (Sodium salt) dissolved in insect ringer solution to obtain 1mM solution. Insect were dipped in solution, excess fluid soaked with bloating paper. Treated insects maintained on fresh leaves.

Insects:

Macrosiphum rosaeiformis (Das) reared outdoors on screened house in potted host plant were used for these experiments. Conditions outdoors was such that only parthenogenetic forms were produced.

Bioassay:-

Post natal effects of juvenile hormone III application.

III and IV instar alate nymphs were collected and sorted into batches. Juvenile hormone III was applied at a dose of approximately 1ug/0.1 ul acetone with the aid of a microlitre syringe capable of dispensing an minimum of approximately 0.1ul; on the dorsal body surface. Control batches received only 0.1 ul of acetone. Treated insects were maintained on fresh rose leaves kept moistened with cotton pads soaked with water. Fresh leaves were changed regularly. The insects were allowed to develop into adult, morphogenesis and mortality was noted down.

Pre- natal effects of Precocene I and II :

Fifth instar and preadult (12-24 hrs) apterous and IV instar were topically treated with a dose of 0.25ug, 0.50 ug and 1.0 ug in 0.1 ul of acetone. Progeny born were allowed to reach third instar stage after which their morph was determined. Percentage of alate and apterous morph was determined after they reached their third instar stage. Crowding of the progeny was minimised by separating them on fresh leaves. Data collected were pooled and percentage of alate forms obtained. Statistical test for significance was done by following student t-test.

cAMP : cAMP was dissolved in insect Ringer solution to obtain a concentration of 1.0mM solution. The solution was applied with the aid of a fine camel hair brush on the body surface.

OBSERVATIONS:

Treatment of juvenile hormone III in microgram dose on the III and IV instar alate nymphs leads to development of morphological aberrations in the developed adults. These responses can be indexed into grades of individuals of increasing morphological responses. Control insects receiving only 0.1 ul of acetone only showed no apparent morphological aberrations, and they were found to develop normally

Morphological Response Index:

Grade 0 - Normal adults without any morphological response.

Grade I - Wings curled at the tips, pigmentation and other features normal.

Grade II- Wings partially curled, other features remaining unchanged.

Grade III- wings totally deformed, abdominal pigmentation lacking, pterothorax reduced.

Grade IV – Crumpled and reduced wings, pterothorax totally unsclerotized and highly reduced in size resembling apterous adult.

Grade V - Supernumerary fifth instar, with large wing pads, unsclerotized pterothorax, and a conspicuous larval wax patches.

Table 3, shows the different percentages of the various morphological grades obtained on treatment of III and IV instars.

Treatment of III instars alate nymphs:

Third instar alates treated with JH-III leads to an increased mortality due mainly to failure to undergo ecdysis, as well as failure in emerging from exuviae. Individuals that emerged into fourth instar survived to produce adults with little to extreme morphological effects; which ranged from individuals with slight curled wings to individuals with total deformed wings,with reduced pterothorax .These higher morphological grades of individuals resembles the apterous form in showing juvenilized features, some individuals underwent fourth moulting to form metathelic fifth instar nymphs (Plate-5,F), these individuals possess nymphal features with extended wing flaps and cuticular pattern remaining nymphal.

Treatment of IV instar alate pre-adult:

The fourth instars treated were in two batches, in batch one only single application of 1micrograme in 0.1 microlitre acetone was made, in the second batch application was done twice at an interval of 48hrs. It was observed that in case of individuals treated with a single dose individuals with morphological response obtained on the lower grades were relatively more, where as in double dose morphological response were more towards the higher grades (Table -3).

Pre- natal effects of P-I and P-II.

Treatment of Precocene-I and Precocene-II at a dose of 0.25μg, 0.50μg and 1.0μg per 0.1 ul of the solvent on the preadult virginoparae does not appear to lead to an increased production of alate progeny. Some percentage of alate forms is born, but is insignificant ($P>0.05$) (Table- 4 & 5). Treatment of fourth instar nymphs also does not appear to enhance the production of alate progeny. (Table –6).

Effect of second messenger (cAMP) in morph determination.

When fourth instar alate as well apterous virginoparous individuals are treated with cAMP at a concentration of 1mM on the general body surface, it

Plate-5: Morphological effects produced by Juvenile hormone-III treatment.

A-Normal alate virginoparae
B-Grade –I
C-Grade-II
D-Grade-III
E-Grade-IV
F-Grade-V

BAR=1mm

Table –3 : Effect of Juvenile hormone –III on the pre-alate individuals.

Quantity of JH-III ug/0.1ul/indv.	No. and instar treated	Mortality after treatment (%)	Percentages of Individuals showing various degree of morphological effects					
			Normal	Grade I	Grade II	Grade III	Grade IV	Grade V
1ug in 0.1ul X1	26 III instar	73.07	57.14	14.28	-	-	14.28	14.28
1ug in 0.1ul X1	51 IV instar	49.01	53.84	23.07	3.84	19.23	-	-
1ug in 0.1ul X2	52 IV instar	46.67	46.6	-	20.00	-	33.3	-

Table-4. Pre-natal effect of Precocene –I treatment on progeny morph

Morph treated	Dose ug/ind	No.of indv. treated	Mortality (%) (Mean±SD)	Total progeny born (Mean±SD)	Total progeny survived (Mean±SD)	Progeny morph (%± SD)	
						Apterous	Alate
Pre-adult apterous virginopara	0.25	20(2)	5.0 ± 0.0	143.0 ± 55.88	80.0 ± 6.36	100.0 ± 0.0	0.00 ± 0.00
,,	0.50	20(2)	0.0 ± 0.0	323.0 ± 26.16	166.0 ± 3.53	95.19 ± 0.81	4.81 ± 0.89
,,	1.0	20(2)	5.0 ± 0.0	192.0 ± 8.48	141.0 ± 28.2	98.5 ±1.52	1.50 ± 0.8
,,	CTL	20	–	175.0 ± 0.00	57.0 ± 0.0	100.0 ± 0.0	0.00 ± 0.00

figure in parenthesis= number of replicates

Table-5. Pre-natal effect of Precocene-II treatment on progeny morph:

Morph treated	Dose ug/indv.	No. of indiv. treated	Mortality (%) (Mean± SD)	Total progeny born (Mean ±SD)	Total progeny survived. (Mean± SD)	Progeny morph (% ± SD)	
						Apterous	Alate
Pre-adult apterous virginopara	0.25	40(2)	17.5 ± 5.65	143.0 ± 22.6	133.0 ± 24.74	98.86 ± 0.8	1.13 ± 0.0
„	0.50	40(2)	15.0 ± 4.07	317.0 ± 116.0	279.0 ± 44.72	100.0 ± 0.0	0.00 ± 0.0
„	1.0	40(2)	35.0 ± 8.13	221.0 ± 28.9	168.0 ± 13.45	98.65 ± 1.90	1.33 ± 0.0
„	CTL	37	21.62 ± 0.0	167.0 ± 0.00	82.0 ± 0.0	100.0 ± 0.00	0.00 ± 0.0

Table-6

Prenatal effect of Precocene -I and II on Progeny morphs of IV instar alate and apterous nymphs.

Morph treated	Dose ug/0.1ul/indv.	Number of individual treated	Mortality	Total progeny born	Total survived	Progeny Morph (%)	
						Apterous	Alate
IV instar apterous Virginopara	0.5ug P-I	40	20.55	292	122	98.37	1.61
,,	CTL	40	12.00	111	40	100.0	0.00
IV instar apterous Virginopara	0.5 ug P-II	90	24.44	362	222	100.0	0.00
,,	CTL	43	18.60	391	277	100.0	0.00

Table-7: Effect of cyclic AMP on emerged adult and the progeny morph of alate and apterous virginoparae

Sl.No	Morph	Number of individuals treated	Mortality (%)	Adults emerged (%)		Progeny morph (%)	
				Normal	Adultoid	W	A
1.	IV alate	60(2)	36.6	100.0	-	-	100%
2.*	,,	60(2)	11.66	100.0	-	-	100%
3.	IV aptera	60(2)	15.0	100.0	-	-	100%
4.*	,,	60(2)	-	100.0	-	-	100%

* Control. W= winged A= Aptera

is observed that the developed adults do not undergo any morphological abnormalities, all individuals developed normally. The progeny born were 100% apterous virginoparae.(Table - 7)

DISCUSSIONS:

Thus, based on the observations it can be inferred that topical application of Juvenile hormone-III causes some degree of apterisation along with juveniulisation in the aphid *Macrosiphum rosaeiformis*. Further, it is also apparent that there exists a difference in the sensitivity of the juvenile hormone depending on the stage of the developing instars treated. Since, it is observed that application of III instar elicits a much higher response than in case on IV instars. The apparent difference in the response to juvenile hormone treatment to each instar stage is probably due to differences in the nymphal age within each instars and individuals that receive the hormone at the sensitive stage thus produce the maximum response. Such differences in the sensitivity of the applied juvenile hormone has also been found in case of the aphid *Megoura* (Lees, 1980). This is further supported by the fact that only JH treated III instar nymphs give rise to the supernumerary fifth instar, treatment of IV instar nymphs even with double dose of the hormone do not lead to supernumerary forms. These observations are commensurate with that of a number of other species of aphids (Lees, 1966,80; Hardie and Lees, 1985).

Juvenile hormone application therefore to the third and fourth instar alate preadult, thus seems to retard the development of wings or alate features, but without completely form reversal. The various morphological grades of individuals obtained resemble the apterous forms in possessing a number of features. The apterous forms essentially differ from the alate in not only in the absence of wings but also in the sclerotisation of the pterothorax, and a lack of marginal sclerite. In the apterous *Macrosiphum rosaeiformis*, the aptera have a light and unpigmented pterothorax with only the pronutum sclerotised, in the alate the pterothorax is well developed with musculature and is highly sclerotised. Treatment of the third instar and with single dose and fourth instar with double dose produced individuals categorised as grade IV in the response index, these individuals had totally reduced and unsclerotised pterothorax. However, single application of hormone to fourth instar did not appear to produce such forms, but; they had wings which were severely reduced. Individuals in the lower grades of the morphological response index had normal pigmentation pattern of the alate, but has varying degree of wing deformity. The production of metathelic forms occurred only on the treated third instars individuals. These individuals after their normal fourth moult developed into supernumerary fifth instar rather than the adult, they are characterised by the presence of juvenilized features. The effects of externous application of juvenile hormone to the developing alate individuals therefore appear to cause juvenilization as well as partial apterization.

Whether juvenile hormone can be attributed to be solely responsible for the differentiation of the alate features during the development of an individual aphid is therefore to be inferred with caution. The production of such morphological grades of individuals with juvenile hormone application has been reported in a number of aphids, but total apterization has not been possible to achieve (Lees, 1966,83;Hardie and Lees, 1985; Nijhout and Wheeler, 1982)

cAMP has been found to have no effects on the progeny morph. Application of cAMP to apterous and alate pre-adult virginoparous individuals have no influence on the progeny morphs under long day photoperiodic conditions, neither does it suppress the development of the alate features when applied to pre-alate individuals. cAMP , therefore does not appear to exert any effect on the morphogenesis in *Macrosiphum rosaeiformis* in a way of second messenger for the juvenile hormone.

The antiallatin Precocene I and II although have been reported to lead to an increase in the alate progeny in a number of aphids (Mackauer,et al ,1979; Hardie, 1986; Hardie, 1986;Rup and Sohal., 1989) however the results are not unambiguous . It is observed that in some it does not lead to the production of alate progeny. (Hardie, 1987).

In *Macrosiphum rosaeiformis* application of Precocene I as well as Precocene II, both, [illegible] does not shows any significant increase in the alate progeny percentage. Since the effects of Precocenes are not fully evaluated in aphids, it needs caution in drawing direct inference from these above observations. In some aphids Precocene have also been found to show a Juvenile hormone effects in the form of precocious adult development and supernumerary moults (Hales and Mittler, 1981;Mittler and Hales, 1984) in the aphid *Myzus persicae* and also metathetely (Hardie, 1986) in case of the aphid *Megoura viciae*.

Fridman-Cohen and Pener (1980) observed that in the insect *Locusta migratoria*, Precocene II treatment to last instar nymphs, induce an effect strictly typical of that of juvenile hormone excess, in spite of exerting the conventional anti-allatin effect eventually It was suggested by them that a supposedly inactive Corpora allata of these nymphs are temporarily activated by the Precocenes, resulting in an outburst of JH biosynthesis before the glands are inhibited. In fact it is reported that low level of Precocene I can stimulate juvenile hormone synthesis in isolated corpora allata (Pratt, 1983). Therefore, it is possible that such action of Precocene will be one reason, whereby, treatment of Precocenes to aphids may not show an apparent increased percentage of alates among the progeny born.

JUVENILE HORMONE , PHOTOPERIOD AND MORPH DIFFERENTIATION

INTRODUCTION:

Photoperiodism is one of the leading factors in the determination of gamic forms of polymorphism in aphids. During winter when environmental conditions are adverse; the parthenogenetic forms of reproduction being inviable are unable to sustain the inclement factors associated with winter conditions, therefore, survival is by the production of sexual forms; namely the Ovipara and the Males; the ovipara is the egg laying form which give rise to the overwintering dipause eggs. The diapause eggs are winter hardy and survive the inclement conditions of winter. The production of these sexual morphs in many holocyclic species are preceded by the sexuparae (or Gynopara) which are the virginoparous forms that arise under the approaching winter conditions of shortening photoperiod and temperature (Lees, 1961,1966; Kennedy and Stroyan, 1959; Hardie and Lees, 1984; Nijhout, 1994).

The sexuparae or the gynoparae that are produced under shortening photoperiod are mainly the alate virginopara that are reproductively different from the long day alate virginopara. It is suggested that these forms are produced by an apparent lowering of the juvenile hormone titre in the haemolymph as a result of a lowered activity of the corpora allata, which is being brought about by the shortening daylength or photoperiod (Hardie and Lees,1985;Hardie,1980).In the aphid *Megoura* the ovipara are produced directly under Short day conditions by the virginopara (Lees, 1959), whereas in *Aphis fabae* the virginoparous form under Short day give rise first to the alate gynopara which further give birth to the egg laying Ovipara and the males (Lees, 1966). In *Megoura* the region of sensitivity to photoperiod have been traced to be the area of the protocerebrum underlying the midline of the dorsum, it was observed that regions other than it did not brought about any significant response (Lees, 1964). Histological examination revealed the presence of a group of 8-12 neurosecretory cells which are arranged in two clusters on either side of the protocerebral cleft in the pars intercerebralis. Radiocauterization of these cells have been found to abolish the photoperiodic response. The virginoparous females under influence of short day, producing ovipara fails to respond to a change to long day condition, and continues to produce ovipara only. Further females producing virginopara under long day switched over to ovipara production (Steel, 1976; Steel and Lees, 1977). This suggests that these cells are responsible for the determination of virginopara under short day photoperiodic conditions. Apart from these cells other groups of neurosecretory cells have also been located, these cells however on cauterisation did not affect polymorphism. It was hence postulated that these so called Gr.I cells regulates the corpus allatum activity by producing an apparent allatotropin named as '*virginoparin*' (Hardie, 1987). Thus under long day conditions a higher activity of the corpora allata leading to a higher titre of juvenile hormone was assumed to be responsible for the control of virginoparous forms, and the gynoparous alate form as a result of diminishing effect of the allatotropin under reduced photoperiopdic stimuli. The involvement of juvenile hormone was further supported by the fact that in the aphid *A.fabae* externous application of juvenile hormone to short day presumptive alate gynopara have been found to mimic the long day apterisation of these morphs (Hardie, 1980). This observation indicates positively that juvenile hormone is involved in the photoperiodic induction of alate gynopara under short photoperiod.

In many species of insects there is a correlation between the cell size and secretary activity (Scharrer, 1964: Panov and Bassurmanova, 1970; Schooneveld et al, 1979). Researchers have used the total corpous allatum volume as an indicator of secretary activity, but it is not always a certain indicator in all species as well as life cycle stages of an insects (Sedlak, 1985).

The activity of the corpora allata has been assessed by the measurement of the corpora allata volume and correlated with various photoperiodic conditions in the aphid *Megoura* (Hardie, 1987;Kats, 1982). Kats (1982) found larger volume of corpora allata in long day *Megoura* in comparison to short day. However, Hardie(1987) on the contrary found a larger corpora allata volume with age under short day in comparison to that of individuals under long day. The activity of the corpora allata is also assessed by the measurement of C/N ration of the corpora allata in a number of insects (Englemann, 1970) in relation to phenomenon like diapause.oviposition etc. Thus an attempt has been made to study the effect of photoperiodism in relation to juvenile hormone, involved in the regulation of alate gynopara production in *Macrosiphum rosaeiformis.*

MATERIALS AND METHODS:

Insects:

The rose aphid, *Macrosiphum rosaeiformis (Das),* used for these experiments were collected from outdoor insect rearing screened house. For the experiments they were maintained on fresh rose leaves (*Rosa sp.*) the petioles of which were kept soaked with cotton pads in a 6 inch petridishes under different required photoperiod.

Bioassay: -

Prenatal effect of juvenile hormone III on the progeny morph of Short day treated virginopara :

Apterous fourth instar (12-24 hrs) individuals were treated with JH-III in doses of 1ug/0.1 µl acetone /individual. Individuals on the control batches received only 0.1 µl of acetone solvent. These insects were subjected to a photoperiod of 8L: 16D at a temperature of 10°C ± 2°C in a temperature controlled BOD incubator. A set of individuals without any treatment was kept under long day consisting of 16L: 8D at a room temp. between 20°C- 24°C, this acted as the Long day control. Progeny born were assessed after they reached the third instar stage.

Corpora allata volume and c/n measurement:-

Fourth instar apterous individuals were treated under short photoperiod of 8L: 16D at a temperature of 10°C ± 2°C, another batch was subjected to 16L: 8D at 20°C ± 2°C. The insects were killed when they reached the adult stage once their parturition was started. The material was fixed in aqueous Bouin's and processed for routine microtomy. 5-6 μm paraffin sections were cut, processed, and stained with Paraldehyde fuschin (Gabe, 1960; Panov, 1980).

The approximate volume of the gland, which is in the shape of a proloid spheroid, was calculated following methods by Srivastava and Verma (1981). Measurement was taken from the serial sections. Volume determined by using the formula $4/3\ \pi\ a.b^2$ (Where a and b are the ½ of the largest and the shortest diameter respectively). Volume of the cytoplasm and nucleus was determined by using the formula $4/3\ \pi . r^3$ (Where,r is the radius of a sphere.) Volume of cytoplasm was obtained by subtracting the total volume with nuclear volume.

Table-8

Juvenile hormone - III and its effect on the progeny morph under Short day on Pre-adult aptera:

Sl.No.	Morph Treated	No. of indv. Treated	Hormone applied ug/0.1ul/indv.	Progeny born (Mean±SD)	Progeny survived (Mean±SD)	Progeny morph (% ± SD)	
						Alate	Apterous
1.	Apterous virginopara	27(2)	1.0 8: 16	76.0 ± 21.65	55.0 ± 13.32	04.12 ± 1.97	95.86 ± 1.0
2.	„ CTL	30(2)	0.0 8:16	183.0 ± 19.07	153.0 ± 26.15	56.82 ± 7.42	43.16 ± 7.4
3.	„ CTL	12 (1)	0.0 16:8	75.0 ± 0.00	58.0 ± 0.00	1.72 ± 0.00	98.27 ± 0 .0

Figure in parenthesis = number of replicates.

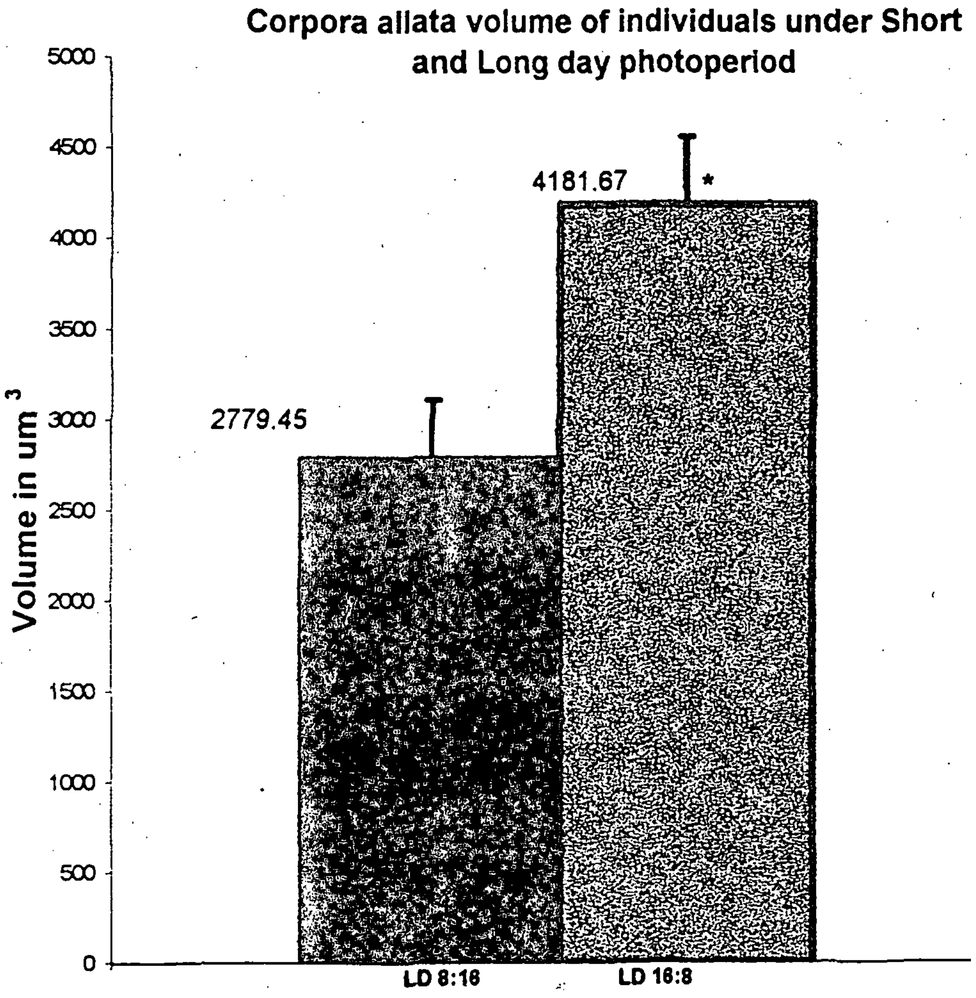
Table-9

Juvenile hormone and its effect on the progeny morph under short day on pre-adult alate.

Sl.No	Morph Treated	No.of indv. treated	Hormone applied ug/0.1ul/indv.	Progeny born (total) (Mean ± SD)	Progeny survived (Mean± SD)	Progeny morph (%±S.D)	
						Alate	Apterous
1.	Alate Virginopara	30 (2)	1.0 8:16	145 ± 33.23	124 ± 18.38	7.06 ± 1.3	92.93 ± 1.3
2.	„	25(2)	- 8:16	65 ± 23.33	53 ± 23.33	13.10 ± 4.3	86.8 ± 4.4
3.	„	10	Control 16:8	132 ± 0.00	120 ± 0.00	—	100.0 ± 0.0

Fig-5: Histogram showing corpora allata volume of individuals under short and long day Photoperiod

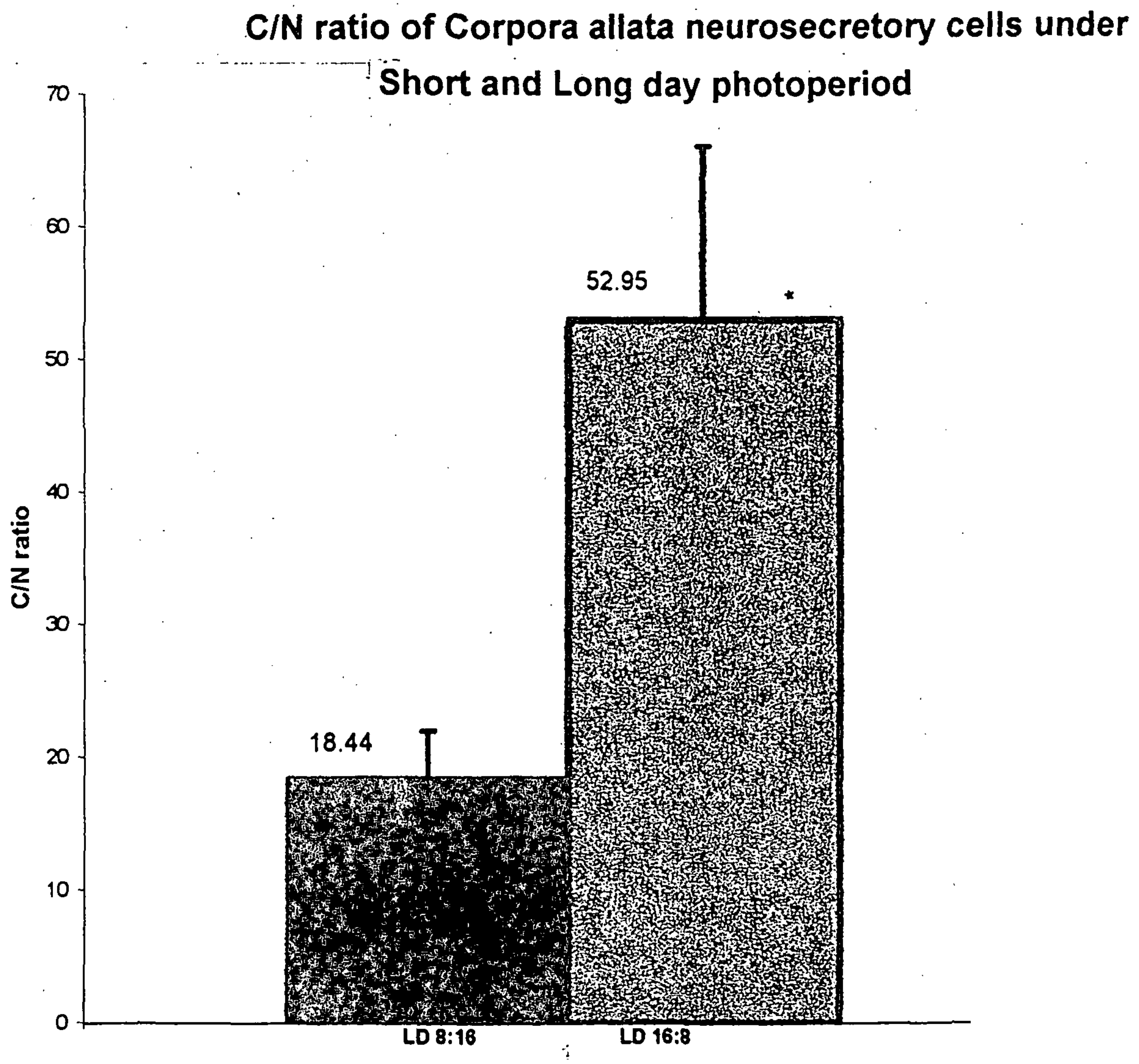
Fig-5



Corpora allata volume of individuals under Short and Long day photoperiod

Fig-6: Histogram showing the cytoplasmic/nuclear ratio of corpora allatal neurosecretory cells under short and long day photoperiod.

Fig-6



C/N ratio of Corpora allata neurosecretory cells under Short and Long day photoperiod

RESULTS: -

Pre-natal effect of JH-III on the progeny morph of short day treated virginopara:

Juvenile hormone -III application at a dose of 1μg/individuals decreased the percentage of alate progeny in the treated batch (4.12 ± 1.42 SD) as compared to (56.82 ± 5.26 SD) in case of Short day, acetone treated control . This is significant at $P < 0.01$, (t-test). Treatment of adult alate virginopara with Juvenile hormone-III appears to decrease the percentage of alate forms in the progeny (7.06 ± 1.32) as compared to (13.10 ± 4.38) control batch under 16L: 8 D at a temperature of 20° C ± 2°C produced as expected a lower alate forms (1.72) in case of apterous and none in case of alates. (Table- 8 & 9).

Corpora allata Volume and C/N ratio:

Corpora allata (CA) volume was calculated from the serial sections. The adult's aphids examined for CA measurement were 2-3 days after parturition had started. Individuals that were treated under short day 8L : 16D and 10°C ± 2°C temp. showed a mean approximate volume of 2779.45μm^3 ± 322.995 SD (n=6); in comparison the CA mean approximate volume for long day individuals is 4181.67 ± 369.98 SD (n=6),(Fig.5) which is significant at $P<0.05$.(t-test). The cytoplasm /nuclear ratio also exhibited correspondingly a higher and a lower value indicating marked differences in the glandular activities. Under long day photoperiod conditions the C/N ratio has a value of (52.95 ± 13.02 SD, n=17) in comparison to that of (18.44 ± 3.13 SD, n=13) in case of the short day treated individuals (Significant at $P<0.05$). (Fig.6)

DISCUSSIONS:

The main objective of this study was to establish the relationship between activity of the corpora allata and consequently the titre of juvenile hormone and the photoperiodic determination of the alate gynoparae. The gynoparae appear during the approaching winter period. They are reproductively different from the alate virginoparae. From the results obtained, it can be safely inferred that Juvenile hormone-III is involved in the photoperiodic determination of alate gynopara in the aphid *Macrosiphum rosaeiformis.* With the approaching winter along with the shortening daylength directly affect the amount of juvenile hormone secreted by the corpora allata of the virginoparous mother, this leads to an apparent decrease in the titre of juvenile hormone, and thus a decreased juvenile hormone level enables the embryos in the parent aphid under short photoperiod to develop towards the alate gynoparous conditions. The gynoparous forms are thus important, for the production of the female sexual egg laying oviparous forms.

The involvement of juvenile hormone is further supported by the activity measurement of the corpora allata under both long and short day conditions. The secretory activity of the corpora allata has been measured by means of the cytoplasmic /nuclear ratio in a number of insects (Engleman, 1970). Baehr,et al (1982) reported that during vitellogenesis the corpora allata size increases in the adult *Labidura riparia* , Khan, et al (1982) measured corpora allata activity in vitro and simultaneously measured CA volume in adult *Leptinorsa decemlineata,*although no correlation was found on spontaneous JH secretion and volume change, however a higher production of JH was found in larger gland than in smaller glands. Tobe and Pratt (1975) have also found a relationship between maximal gland activity and CA volume.

In aphids, the activity of the CA has been correlated with the alate/apterous conditions. White, (1966) measured the volume of the corpora allata in the alate and the apterous individuals of the aphid *Aphis fabae* , and found a reduced corpora allata volume in case of the developing alate nymphs to that of the apterous. Kats (1980) measured the volume of the corpora allata between the individuals treated under short and long day photoperiod, and found that corpora allata volume under short day decreases initially and then increases in the short day forms, where as in case of the long day the corpora allata volume increases significantly.

The activity as indexed by the Cytoplasmic/nuclear ratio of the CA neurosecretory cells as well as the approximate volume of the CA shows significant variation. A higher C/N ratio along with a larger volume of CA can thus be implicated to be responsible for enhanced activity of the corpora allata during long day conditions, whereas a reduced C/N ration and volume during short day conditions implies a lowered activity of the corpora allata.

Studies on the correlation of the activity of the corpora allata with morph determination in other species of aphid, have also been found to have similar relationship. In *Brevicoryne brassicae*_(L), it was found that the size of the corpora allata in the apteriform fourth instar nymphs is larger than that of alatiform aphids. But, after the imaginal moult the size doubled in the alatiform, where as the CA size was reduced in the apteriform adult individuals. It was thus suggested that the large size of CA in the alatiform adults is at a higher activity and thus, a higher level of juvenile hormone is secreted. This leads to the exclusive production of the apterous progeny, further the lower size of CA in the apterous forms was supposed to have less effect on the progeny morph, and it could be influenced mostly by the environmental factors (White, 1968).

Thus, a higher activity of the corpora allata in the long day individuals of *Macrosiphum rosaeiformis* can be implicated as a determinant in the differentiation of the apterous forms. Whereas, under short day a lowered corpora allata activity leads to the production of alate gynoparae

NEUROSECRETION

INTRODUCTION: -

Neurosecretory cells are those nerve cells that *'specialise in the manufacture of chemical mediators to a degree greatly surpassing that of the more conventional neurones and that have a secretary activity comparable to that of gland cells"* (Scharrer, 1977). They occur in all ganglion of the central nervous system and the brain contains the largest numbers of them. The brain neurosecretory cells occur in several clusters with distinctive location.

Although all neurons are secretory in the sense that they secretes small quantities of neurotransmitters, for e.g. acetylcholine, glutamate, etc. which are used in the transfer of information from one nerve cell to another. On the other hand neurosecretory cells possess large amount of chemicals called neurohormone into the circulating system and are carried to the target cells or sites, where they bring about some physiological activity.

In insects neurosecretory hormone control a number of process, which includes Ecdysone synthesis by prothoracicotropic hormone secreted by the median and lateral neurosecretory cells of the brain (Wigglesworth, 1940; Agui et al, 1979;Williams, 1948; Steel, 1978).

Juvenile hormone synthesis by allatotropin in case of *Manduca* and *Diploptera* (Bhaskaran et al, 1980; Tobe, and Stay, 1980) and also the regulation of polymorphism of aphids which is believed to be regulated by an apparent virginoparae inducing hormone as observed in case of the aphid *Megoura,* secreted by the Group I brain cells (Steel and Lees, 1977). Apart from these they are also involved in Diapause of eggs (Kabota, et al, 1977), pupariation (Zdarek, et al., 1981), cuticular tanning by Bursicon (Truman, 1973; Vincent, 1972; Mills, et al., 1965;Fraenkel, et al., 1972), Eclosion behaviour (Truman. et al., 1981), Ovulation (Kriger, 1981), Haemolymph sugar levels (Steele, 1961), Protein synthesis in fat bodies (Carlisle and Longhton, 1979), Fluid secretion by Malpigian tubules (Maddrell,1963), Fluid secretion by Malpigian tubules and rectum (Steele and Tolman,1980),Absorption from rectum (Phillips et al., 1980), Heartbeat (Gersch et al., 1970).

Recognition of neurosecretory cells by light microscopy has been done by utilising selective stains like Paraldehyde fuchsin Chromehaematoxylin phloxine, etc., these techniques have been used extensively in insects (Raabe, et al., 1979;Panov, 1980). These selective stains can be used as a first indication that a cell may be neurosecretory and further it also aid in tracing the axon process carrying the neurosecretory material to the target cells and releasing them via the release site or the neurohaemal organ.

The identification of these neurosecretory cells also depend upon the numbers of staining techniques used and as well as the physiological state of the cell (Panov, 1980), the use of electron miscroscopy has revealed a common substructure of NSC on many occasions. These cells revealed the possession of numerous elementary neurosecretory granules with a diameter of 100-300nm and are of electron dense in nature.

The morphology of insect neurosecretory cells is not very different from the usual neurons. It consists of a cell body with processes consisting of dendrites and axons. They may be either mono or multipolar. The specialisation of neurosecretory cells takes place at the axon terminal, which divide into many fine branches terminating into a swollen ending containing large amounts of neurosecretory granules. These terminals constitute the region called the neurohaemal site or the site of release of the neurosecretory material.

The secretary products of the neurosecretory cells are proteinaceous as well as amines which act as neurohormones and are released in the circulatory system. Based upon their content, the staining affinity of these neurosecretory cells differ. They are usually classified as ' A' and 'B' cells based on their staining affinity with stains like Paraldehyde fuchsin(PAF), Chromehematoxylin pholoxine(CHP) etc., 'A' cells are present in the pars -intercerebralis of the protocerebrum and other region of the nervous system, they stain intensely with paraldehyde fuschin (PAF), performic acid-Alcian blue (PAB), Chrome-haematoxylin phloxine (CHP) after suitable fixation, indicating the presence of sulphuric rich protein. This has been confirmed by a number of workers (Panov, A., 1980; Rowell.1976). The presence of high sulphuric content in invertebrate neurosecretory cells in not however clearly understood. The 'A' cells in the pars-intercebralis of insects have been shown to be implicated in the control of oogenesis, ovulation, protein synthesis, diuresis and colour change. The staining properties of the material in the neurosecretory cells are most probably due to the common molecule or precursors rather than the hormone itself. The 'B' cells on the other hand has less affinity with Paraldehyde fuchsin, Chromehematoxylin , but stains strongly with Phloxine .(Orchard and Loughton,1985).

In aphids neurosecretary cells are distributed on the brain, sub-oesophageal and the abdominal ganglia. Johnson, B (1962; 1963) on the basis of his study of neurosecretion in several species of aphids, found that the neurosecretory cells in the brain of aphids carry neurosecretory material over to long distances through axonal transport.

The photoperiodic receptor responsible for the photoperiodic control of morph determination in the aphid *Megoura viciae,* have been found to lie on the region of the protocerebrum underlying the midline of the dorsum (Lees, 1964). Histological studies have revealed the presence of a prominent group of around 8-12 neurosecretary cells arranged in two clusters on either side of the protocerebral cleft in the pars-intercerebralis. These cells were considered homologous to the median neurosecretory cells in other groups of insects (Johnson,1963; Gabriel, 1965;Steel, 1977)

Steel (1976, 77), Steel and Lees (1977) and Steel (1978) mapped the neurosecretory cells present in the brain and studied their role in the photoperiodic regulation of polymorphism by employing specific staining techniques with Paraldehyde fuchsin and Chromehaematoxylin phloxine

In the aphid *Megoura*, the group -I cells activity have been corellated with morph differentiation under short day photoperiod (Steel, 1976;Mittler et al, 1979;Lees, 1980).

Thus, attempt is made to map the different neurosecretory cells in the brain of the aphid *Macrosiphum rosaeiformis*, and to understand the role played by the protocerebral neurosecretory cells in relation to photoperiodism. Further, experiment is also conducted to study the neurosecretory cells in the corpora allata in relation to photoperiodism.

MATERIALS AND METHODS:

Staining of neurosecretory cells was done by methods as mentioned by Gabe (1966) and Panov (1980).

Preparation of Paraldehyde fuchsin (PAF): -

1gm of basic fuchsin is added to 200ml of boiling water, boiled for few minutes cooled and filtered. To the filtrate, 2ml each of concentrated HCl and paraldehyde was added. This was left stoppered at room temperature. The solution was filtered after 3-4 days when it lost the reddish colour. The precipitate on the filter paper was dried at 50°C; the dry crystals are then removed from the filter paper and stain prepared.

Preparation of the staining solution-

0.5 gm of the crystals so obtained was dissolved in 50ml of 70% alcohol and allowed to dissolve. To this 1ml of glacial acetic acid was added to obtain the final staining solution.

Staining procedure:

1. Slides dewaxed in xylene.
2. Sections dehydrated in descending grades of alcohol 100% -

30% to water.

3. Section Oxidised with fresh mixture of $KMnO_4$ solution containing equal volume of 0.6% $KMnO_4$ and 0.6% H_2SO_4 for about 1 minute until section attain a tobacco brown colour.
4. Rinsed in distilled water,and bleached in 2.5% Sodium metabisulfite solution for 20- 40 seconds.
5. Washed the section in running water for 5-15 minutes.
6. Dehydrated in alcohol series till 70% and stained in PAF solution for 5 minutes.
7. Differentiated in 95% alcohol.
8. Cleared and mounted in DPX.

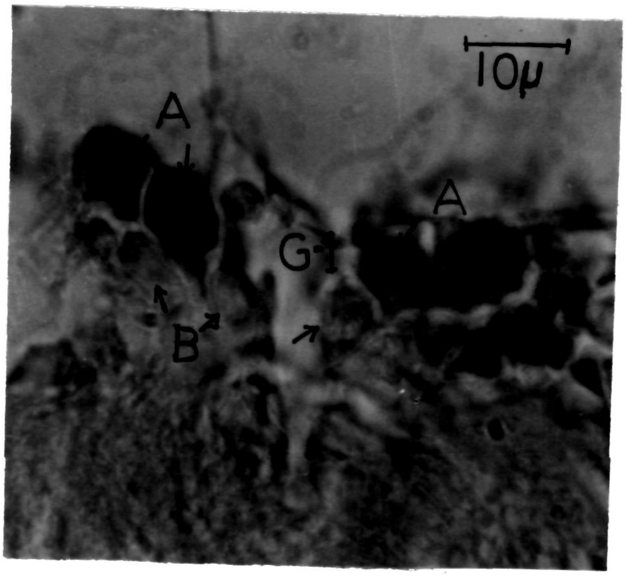
For the study on the effect of photoperiod on the neurosecretory cells, fourth instar apterous virginoparous individuals were subjected to a short day photoperiod of 8L : 16D and temperature of 10°C ± 2°C in a BOD incubator. A Control batches of insects were kept under a long photoperiod of 16 L : 8D at a room temperature of approximately 20°C ± 2°C. They were killed once parturition was started and fixed in aqueous Bouin's solution. Embedding was done in paraffin wax and routine histological techniques employed to obtain 5um - 8um sections. Sections were stained with Paraldehyde fuchsin as mentioned before.

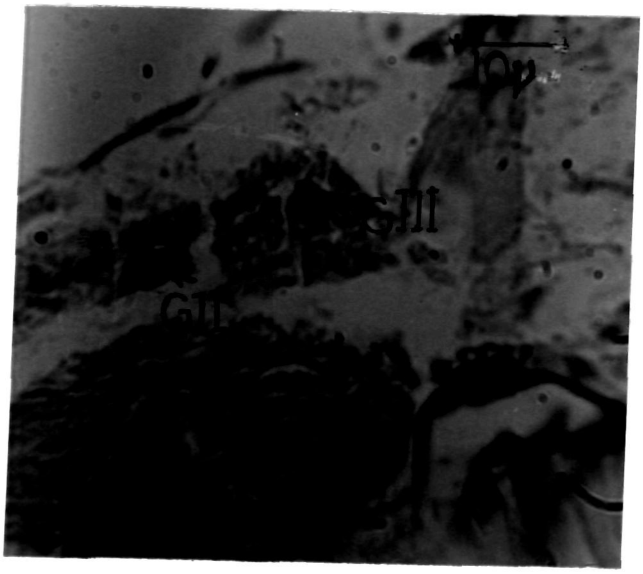
OBSERVATIONS:

Histological studies reveal the presence of groups of neurosecretory cells localised in the brain. The distribution of neurosecretory cells in the brain is similar to that of other insects. The neurosecretory cells in the brain, parallel to other insects consists of the median and lateral cells.

In *Macrosiphum rosaeiformis* four groups of neurosecretory cells are localised, namely Group I to group IV (Plate-6; Table-10). They stain selectively with Paraldehyde fuchsin. The prominent groups of cells among these are the Group I cell located on the anterior protocerebrum on either side of the protocerebral cleft. This group consists of around 4-5 cells. There appears to be a difference in the staining reactions among these cells. It is observed that the anterior larger cells stain deeply with paraldehyde fuchsin and around 1-2 cells in the posterior region however shows very light staining property. The strongly paraldehyde positive cells in the anterior most region are larger, they are observed to be octagonal in shape with many dendrites. The nucleus is centrally located and the axons appear to be directed towards the central brain mass, however, these axons could not be traced for a longer distance.

Plate-6: Plate showing the various groups of neurosecretory cells in the Brain.


10μ
A
A
G·I
B


10μ
G.III
G.II

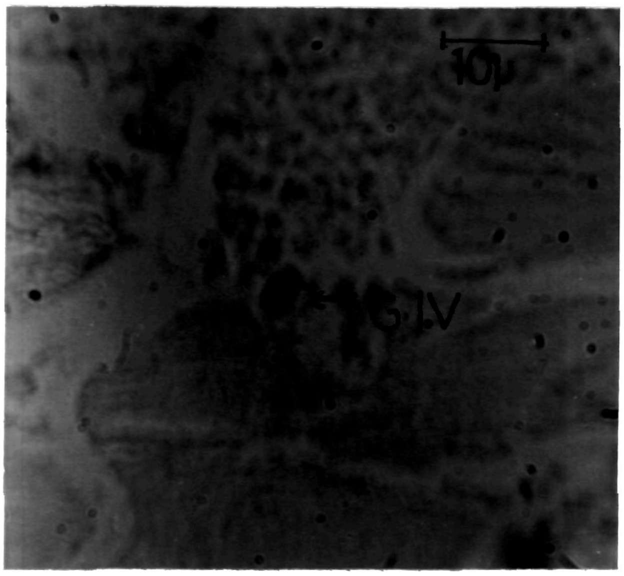

10μ
G.IV

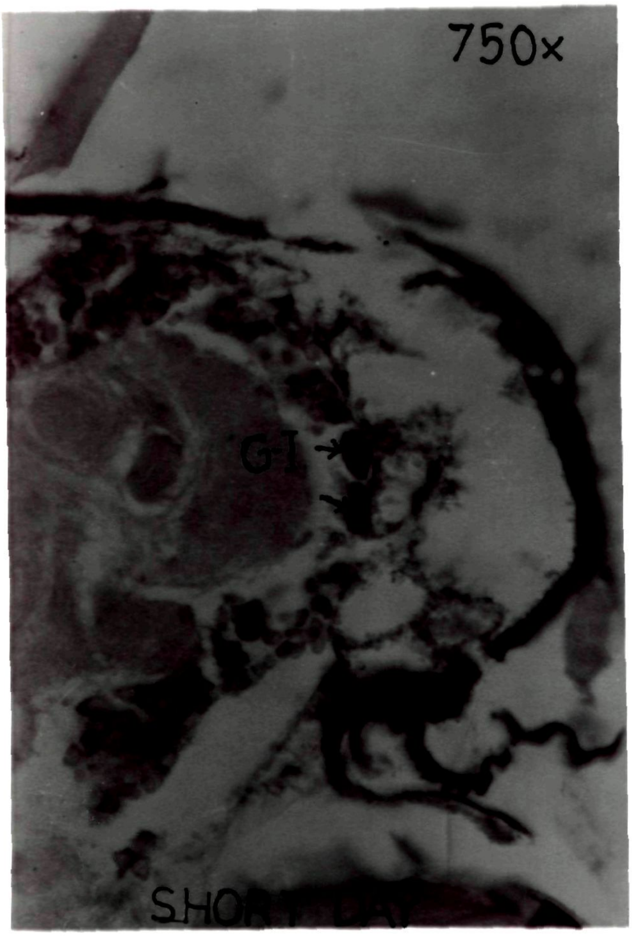
Table-10: Characteristics of the different groups of brain neurosecretory cells

Table-10

	NSC Groups			
	Gr.I	Gr.II	Gr.III	Gr.IV
L.A in µm (range)	12.04 (7.5 –20.0)	15.00 (11.25-17.5)	16.75 (13.75-22.5)	15.0 (13.00-17.50)
S.A in µm (range)	8.40 (5.0-12.5)	11.45 (10.0-15.0)	10.57 (6.25-15.0)	7.5 (3.50-10.50)
N diameter in µm.	5.56 (3.75-12.5)	6.87 (5.0-9.5)	6.65 (3.75-10.0)	5.0 (3.50-10.50)
Number of cells and localisation	3-4 On either side of protocerebral cleft	1-2 Behind protocerebrum	1-2 Lateral and behind Gr.II cells.	3 Junction between Optic and the protocerebral lobe
Shape	Elongated oval to irregular	Teardrop shaped	Varies from oval to round	Elongated oval to polygonal
Type and Staining affinity	A , B +++, +	A +++to++	A +++to++	A +++to ++

L.A=long axis,S.A=short axis,N=nuclear,+++=intensely stained, ++=medium stained,+=little or no staining.
(All measurements done with the aid of stage and ocular micrometer .No of cells n = 5-6)

Plate-7: Plate showing the effect of photoperiod on the Group -I neurosecretory cells


750×
G I
SHORT DAY

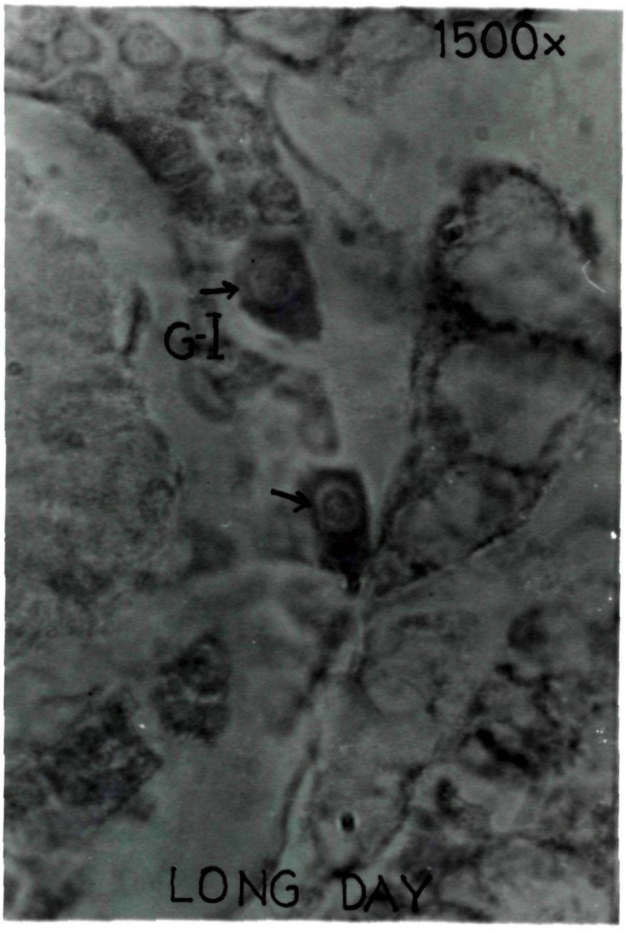
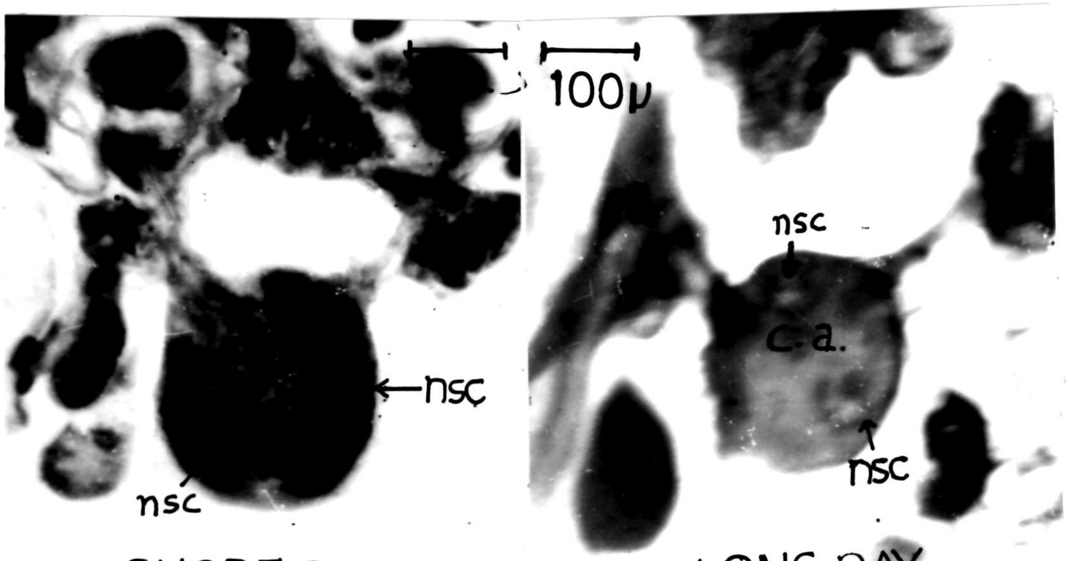

1500×
G-I
LONG DAY

Plate-8: Plate showing the effect of photoperiod on the corpora allata neurosecretory cells.



SHORT DAY

LONG DAY

Plate-9: Plate showing the distribution of neurosecretory cells in the brain.

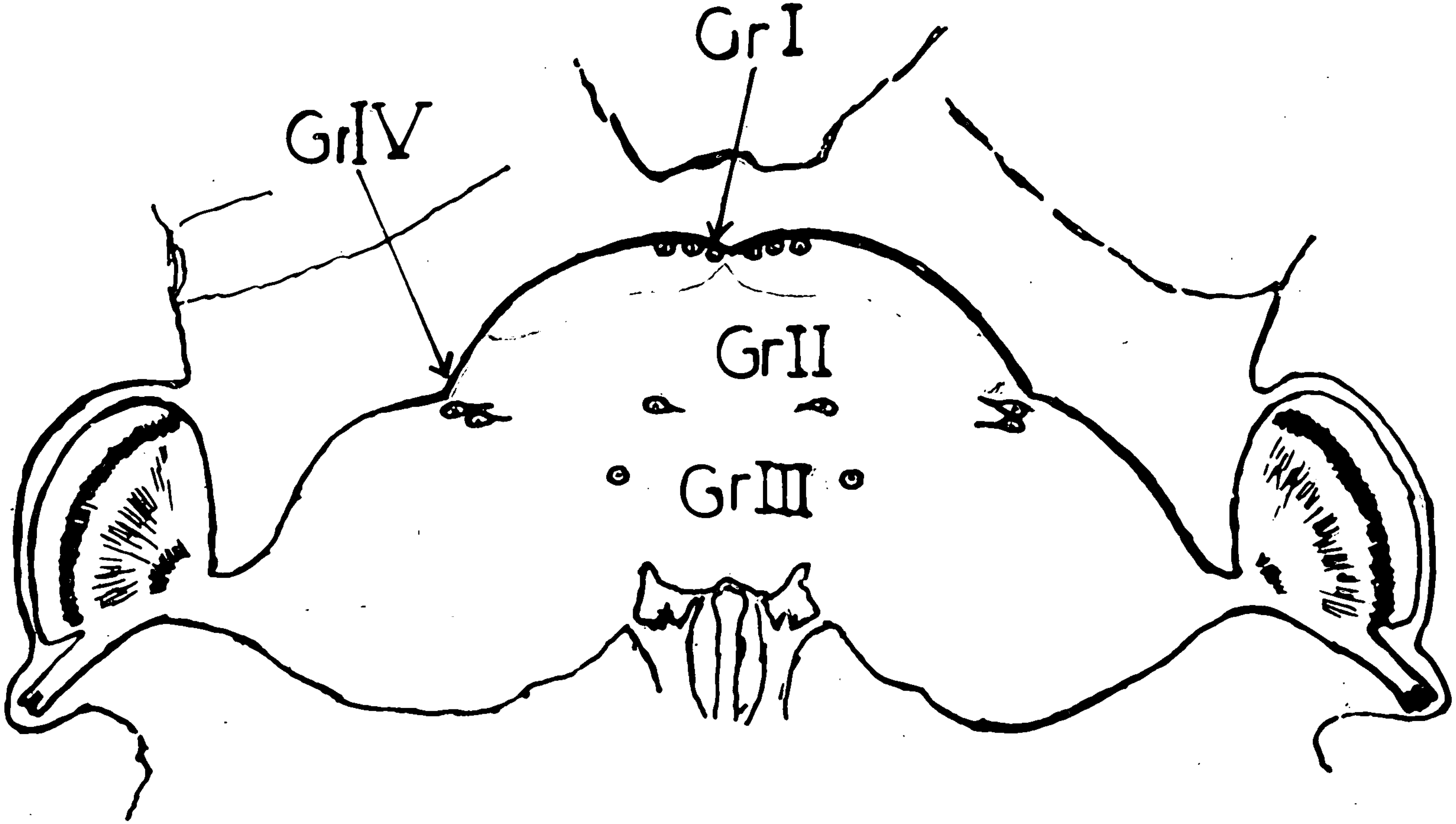


Plate - 9

Schematic diagram showing the location of various groups of brain neurosecretory cells

According to the staining affinity of these cells with PAF, they have been found to be of two types, viz. 'A' type, staining strongly with PAF, and 'B' type, staining weakly with PAF. (Plate-6)

The Gr I cells shows differences in the positive neurosecretory content of individuals under short and long day conditions. A relatively more PAF positive material is observed in case of the short day treated insects than in case of the long day. (Plate-7).

The corpora allata in *Macrosiphum rosaeiformis* is a single proloid spheroid body located posterior to the brain mass and lies in between the corpora cardiaca. Many neurosecretory cells are observed, they show differences in their affinity towards PAF stain. Under short photoperiod the Paraldehyde fuchsin positive granular contents is more in the neurosecretory cells as compared to that of long day photoperiod. (Plate-8)

DISCUSSIONS:

Lees, (1964) first of all studied the location of photoperiodic receptors in the aphid *Megoura viciae* Buckton, in relation to the phenomenon of polymorphism. He observed that the region anterior to the brain is the most sensitive to photoperiodic treatment.

Histological studies have shown the presence of a prominent group of around 8-12 neurosecretary cells arranged in two clusters on either side of the protocerebral cleft in the pars-intercerebralis. These cells were considered homologous with the median neurosecretory cells in other groups of insects (Johnson,1963; Von Gabriel,1965;Steel,1977). In the aphid *Megoura viciae,* the group -I cells shows a presence of a large quantity of neurosecretory material in the perikaryon under the conditions of short day photoperiod, whereas in case of long day photoperiod the quantity of neurosecretory material decreases. When these cells under long day conditions were ablated it was observed that the individual immediately revert to production of the sexual forms, further individuals that were producing oviparae failed to respond to a change over to long day photoperiod. Thus, it was suggested that a factor called 'virginoparin' is produced that acts on the ovaries and promotes then to develop into virginoparous forms. Whereas, there absence during shortday causes the production of sexual forms. (Steel, 1976;Mittler et al, 1979;Lees, 1980).

The localisation and numbers of the various groups of neurosecretory cells in the brain of *Macrosiphum rosaeiformis* appears to correspond with that of *Megoura* as described by Steel (1977). However, the group V cells corresponding to the group IV in *Megoura* as described by Steel was not observed and thus appears to be lacking, as no specific cells corresponding to this group were observed. Johnson (1963) described the neurosecretory cells in the brain of a number of species of aphids including *Aphis fabae*, where only group-I and group- II cells in the brain have been described. Pare et al (1974) also studied the ultrastructural features of the neurosecretory cells in the brain of the aphid *Macrosiphum euphorbiae*, a total of only three groups have been mentioned.

The neurosecretory cells in the brain are relatively few and varies between 2-3 in each group. The Group I cells are however more in numbers, consisting of atleast 4-5 cells on either side on the protocerebrum. Morphologically, these cells differ, some multipolar cells is observed that stains deeply with PAF, the axonal terminals of which are directed towards the neurophyll.

The neurosecretory cells in the corpora allata shows different staining affinity with PAF under long and short day photoperiodic conditions. This suggests that the activity of the corpora allata vary between long day and short day photoperiod.

Differences in the secretory activity as revealed by the PAF positive material in the cells under different photoperiod thus, suggests that the activities of these cells are directly influenced by the photoperiodic conditions. Therefore, these cells appear to be involved in the mediation of the photoperiodic control of polymorph differentiation as in case of the aphid *Megoura viciae*. It is probable that the regulatory mechanism might involve the production of neuroendocrine hormones, which directly regulates the secretory activity of the corpora allata.

APHID CONTROL

INTRODUCTION:

The inherent problems like toxicity, persistence and degradation have given rise to the lookout for newer and less deleterious agents for the control of insects pests. The potential use of insect's own hormones as a means of insect control was therefore held promising. Williams (1967) coined the term '*Third generation pesticides*' for such agents.

The most potentially utilised third generation pesticides constitute the Juvenile hormone Analogues and anti-allatin that are recently being evaluated. Natural juvenile hormone has been found to be species specific and to be extremely unstable in the environment and extraordinarily difficult and expensive to synthesise in quantity. This had lead to the development of JH analogues like Kinoprene, Methoprene etc. that are now commercially available and are licensed as insecticides. JH analogues or mimics are more potent than natural JH and act by disrupting reproduction and act as birth control agents (Retnakaran et al., 1985).

However, the idea that JH compounds and analogues as insecticides could not lead to development of resistance in insects against this compounds were dispelled when it was found that insects develop a resistance to JH analogues just as rapidly as they do to any other type of insecticides (Hammock, 1985;Hammock et al., 1977; Plapp and Vinson, 1975). Further these JH insecticides are effective only during a specific period in the life of an insect; during which it is not sensitive and cause the disruption in metamorphosis, further the half life of JH analogues are so short, so as to cause any effective control in single use in an asynchronous population of pest insects.

Another option that was looked as a means of control insect pests more effectively than that is possible by JH analogue was through a possible anti-allatin compounds; which will destroy the corpora allata at an early stage of the insects. Search for such compounds had lead Bowers et al (1976) to discover the compound Precocenes from the plant *Ageratum haustonianum*. Assay performed on the milkweed bug *Onchopeltus was* found to cause precocious metamorphosis. Two compounds Precocene-I and Precocene-II were obtained on purification of the crude extract of the plant. These compounds when applied to insect's leads to a number of developmental and physiological defects in mainly hemimetabolous insects. These effects include the inhibition of ovarian development, premature metamorphosis and other morphogenetic defects.

The use of these anti-allatin compounds such as Precocenes for control could work by the inhibition of proliferation and production of morphological aberrant forms. Effective control strategies require a check of insect infestation as well as the prevention of transmission of viral diseases by the alate individuals of a population.

Damage and destruction of crop plants by aphids attack takes place at different levels, firstly deterioration of plant quality by the increased infestation and proliferation and secondly the appearance of alate forms which migrates to newer food source and thereby carrying the pathogen including viruses that causes spread and transmission of diseases.

The effects of Precocenes on the developing morph forms both apterous and alate is not fully evaluated in aphids. In the aphid *Lipaphis erysimi* precocene-II treatment produced both toxic and anti-juvenilsing effects. In the young first instar individuals it resulted in the inhibition of emergence, in adults it leads to reduction of fecundity and longevity (Rup and Sohal, 1989). In *Aphis craccivora* Precocene-II treatment leads to a reduction in the progeny number, survival and life span of the adult, apart from these precocious metamorphosis is also noticed in the developing nymphs (Srivastava and Jaiswal, 1989).

In *Myzus persicae* treatment of the parthenogenetic forms with the Precocene analogue 7-ethoxy-6-methyl-2, 2-dimethyl chromene induced precocious metamorphosis in the progeny, they become adult in the third or fourth instar (Hales and Mittler, 1981). Further, there are also reports where Precocene treatments in many insects' species have lead to production of juvenile hormone agonist effect. In *Megoura viciae*, precocene treatment has been found to cause metathetely (Hardie, 1987). Precocene-III which is more potent than precocene-II have been found to cause supernumerary moults in *Myzus persicae* (Hales and Mittler, 1983; Mittler and Hales, 1984). Supernumerary forms have been found to occur in *Acrythosiphum pisum*, *Aphis fabae* as well as *Megoura viciae* by similar treatments (Hardie, 1986).

These anti-allatin compounds are thus, mainly used extensively in experimental studies on the role of JH in development and reproduction as well as in physiological and behavioural, but have not been applied to the problem of control of Insect pests (Nijhout, 1994).

'Queen substance' (9-oxodec-trans-2-enoic acid) is secreted by the queen honeybee, it is taken up by the workers bee. In sufficient quantity the queen substance inhibits the development of the ovaries in the worker female and the production of further queens. Apart from its effect on the physiological, behavioural affects in bees, it also has a similar effect on other groups of social insects like *Formica fusca* L,(Carlisle and Butler, 1956) and the termite *Kalotermes flavicollis* F. (Hrdy et al,1960) and in the house fly *Musca domestica* L. (Nayar, 1963). Queen substance has been found to be innocuous to the immature stages of *Aedes aegypti* L, except during the larval and pupal moult. A delayed toxic effect was observed, treated honeybees sickened and died during the pupal stage (Sayeed Qurashi and Thorsteinson, 1965). However, reports on the ovicidal effects of queen substance in aphids are lacking. Therefore, an attempt has been made in the present study to evaluate the efficacy of queen substance in the aphid *Macrosiphum roseiformis.*

MATERIALS AND METHODS:

Chemicals: Precocene - I and Precocene -II used were obtained from Sigma –Aldrich USA. Required quantity of these compounds was dissolved in spectrum grade acetone to obtain varying concentration of the compounds.

Application of these solutions was through a microliter syringe. Specified dose of the compounds per 0.1ul (approximate) of acetone was applied on the thoracic region of the developing pre- alate IV instar nymphs. Treated insects were maintained on the fresh leaves kept moistened with cotton pads in petridishes. Control insects received only acetone treatment.

Mortality and morphogenesis were recorded regularly during the course of the experiment. Photoperiod was 12L: 12D, Light: Dark and temperature 20°C ± 2°C.

'Queen substance' (9-oxodec-trans-2-enoic acid) obtained (Glaxo) was in acid form. It was converted into neutral salt. The compound was neutralised by dissolving it in a solution containing 50 ml ethanol, to this solution drops of 0.5 % KOH solution was added and pH measured with a pH paper. Addition of KOH solution is stopped once the solution attains neutral pH (pH 7). The solution was then evaporated to dryness in a water bath, the salt obtained is weighted, and 1 mg of salt was dissolved in 500µl of Insect ringer solution. Application of this solution is done by spraying it on the leaves where individuals were allowed to feed.

Plate-10: Plate showing the morphological aberrant form produced on treatment with precocenes.

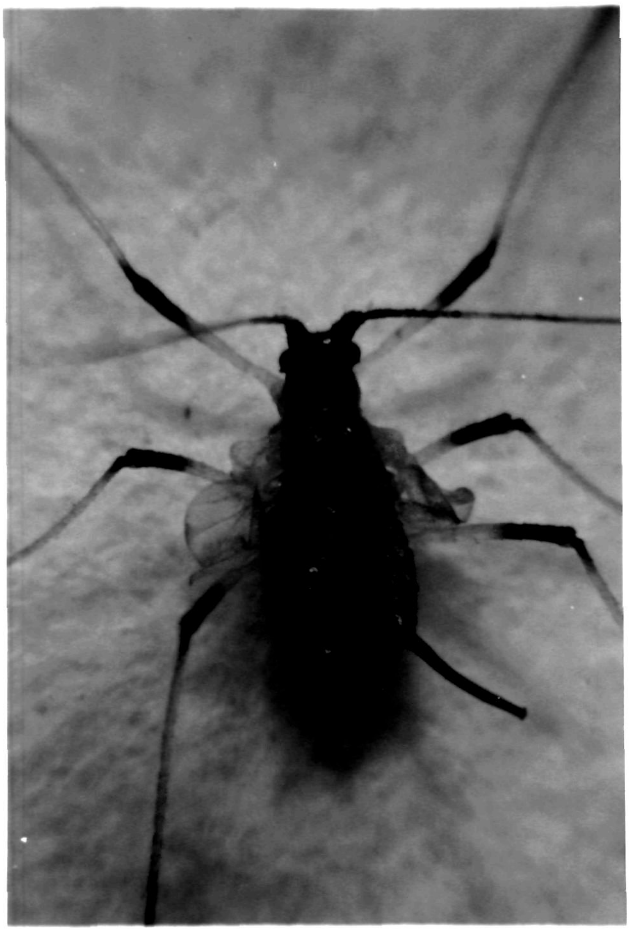


Table-11

Effects of Precocene-I and Precocene -II on pre-alate nymphs

Sl.No		Dose (ug/0.1ul/indv	No.of indv. treated	Mortality (%)	Morphological aberrations (%) in Grades				
					N	G1	G2	G3	G4
1.	Preco-I	6.25	50	-	96.0	2.0	-	2.0	-
2		12.50	90	11.0	86.0	-	3.0	-	1.0
3.		25.0	36	61.1	35.7	14.2	42.8	7.1	
4.		50.0	34	67.6	26.4	43.4	30.43	-	-
5.		CTL	55	-	100.0	-	-	-	-
6.	Preco-II	1.25	55	21.81	74.54	4.65	-	-	-
7.		2.50	30	20.0	41.66	20.83	33.33	-	-
8.		5.00	46	32.	32.25	19.35	22.58	25.80	-
9.		CTL	26	19.23	100.0%	-	-	-	-

N-Normal individuals, G_{1-4}- morphological grades.

Fig-7: Histogram showing the various grades of morphological aberrant forms produced on treatment of precocene-I on pre-alate nymphs.

Fig- 7: Effects of precocene-I treatment on pre-alate nymphs.

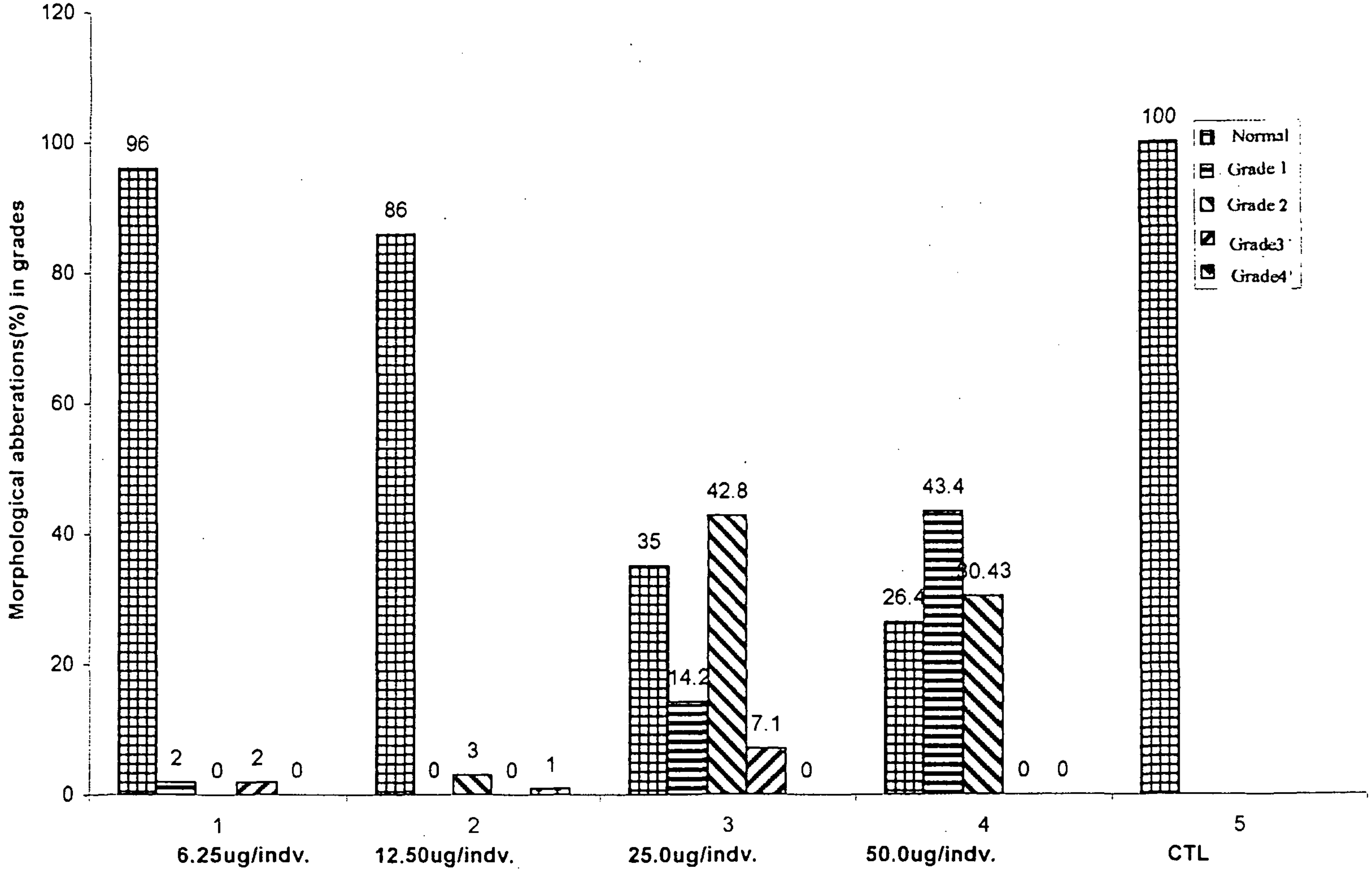


Fig-8: Effects of precocene-II treatment on pre-alate nymphs.

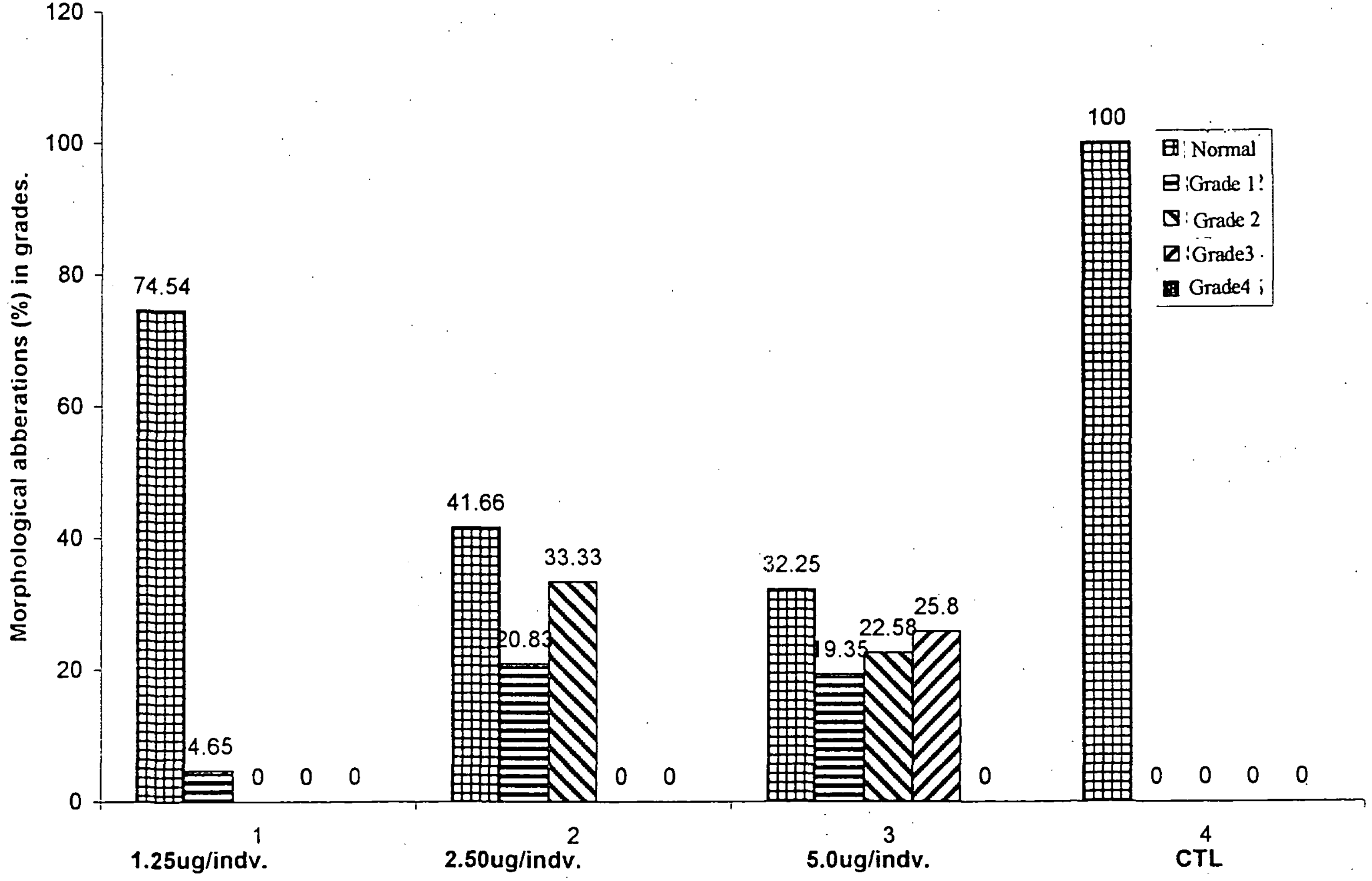


Table-12

Effects of Queen substance (9-0xodec-trans-2-enoic acid) on parturition of alate and apterous virginoparae.

Morph treated	Dose applied	Number of individuals treated	Mortality after treatment	Progeny born /individual (Mean± SD)	Percentage of individuals	
					With progeny	Without progeny
IV apterous Virginoparae (12-24hrs)	1mg/500ul	26	0.0	9.0 ± 6.20	34.61%	65.32%
IV apterous Virginoparae (12-24hrs)	Control	12	0.0	16.88 ± 11.62	75.00%	25.0%
IV alate Virginoparae (12-24 hrs)	1mg/500ul	38	0.0	3.83 ± 1.80	26.08%	68.42%
IV alate Virginoparae (12-24hrs)	Control	12	0.0	21.33 ± 8.72	100.0%	0.0%

OBSERVATIONS :

Effects of Precocene I and Precocene II:

Precocene I and II when applied at a dose of 6.25μg, 12.50μg, 25.00μg and 50μgms per individuals to the early alate instars leads to the productions of varying percentages of morphologically deformed individuals.Precocene-II however was effective at a dose of 1.25 μg, 2.50 μg and 5.0 μg /indv. The effects produced by precocene-I and II includes the increased mortality and severely deformed appendages. Wings are highly curled and reduced in size, the legs showing unusual curling (Plate-9). The various morphological aberrant individuals can be graded according to the severity of the effects produced. (Table-11). Further, it is observed that with an increase in the dose, an increase in the effects are observed with increasing dose.(Fig-7 & 8).

Effects of the Queen substance:

'Queen substance' when applied to individuals leads to a significant reduction in the number of progeny born as compared to the untreated control. In case of apterous virginoparae the mean progeny born per individual is 9.0 ± 6.2 (SD) as compared to the control, which is

16.88±11.62(SD), (significant at $P<0.05$), in case of the alate virginoparae the mean number of progeny born per individual in the treated batch consists of 3.83±1.80(SD) as compared to 21.33±8.72(SD), (significant at $p<0.01$) in case of the control. Apart from its effects on the parturition, no other morphological effects were observed. (Table- 12)

DISCUSSIONS:

From the above experiments it is clear that the anti-allatin Precocene-I and Precocene -II causes severe morphological abnormalities in the developing alate instars. The cause may be due to a number of factors, the foremost of which may be due to hormonal disturbance. It is reported that anti-allatotropin like Precocene induces mainly symptoms of juvenile hormone deficiency in some sensitive insects belonging to heteropterans (Bowers et al, 1976, Unnithan and Nair, 1974, Alrubeai, 1986).

The morphological aberrant form clearly demonstrates the developmental interruption by inducing changes in phenotypic abnormalities during the coarse of development of an individual during metamorphosis, moulting, and morphogenesis.

Precocenes, which acts by inhibiting the activity of the Corpora allata, affects the Juvenile hormone controlled processes of development such as moulting and metamorphosis in many insects. (Azambuja,et al, 1981; Deb and Chakravorty, 1985; Mukopadhyay et al, 1988). Destruction or degeneration of corpora allata after treatment with Precocenes may cause the decrease in the level of the developmental hormone viz. the juvenile hormone, however, the effects produced by the precocene treatment observed in case of the alate instars of *Macrosiphum rosaeiformis* shows similarities with that of individuals treated with juvenile hormone, thereby showing results which is contrary to the effects produced by Precocenes. This may be explained as due to an apparent antagonistic effects of Precocene as reported in the insect Locusta migratoria (Fridman- Cohen and Pener,1980).It was observed that in this particular insect's application of Precocene leads to an apparent juvenilising effects, which was explained as due to an apparent increase in the activity of the corpora allata prior to their destruction by the action of Precocene.

The action of the 'Queen substance' also appears to be effective in inhibiting the process of embryogenesis and thereby leading to a decrease in parturition. Although the exact mechanism of action of 'Queen substance' is not studied, it may be possible that it acts on the developing embryo and inhibits their full development. Therefore, the action of 'Queen substance' on *Macrosiphum rosaeiformis* shows its potency as an agent in controlling the proliferation of aphids. Moreover, its action on this aphid corroborates similar effects observed in case of social insects like *Formica fusca* (Carlisle and Butler, 1956), *Kalotermes flavicolis* F. (Hrdy et al, 1960) and in case of *Musca domestica* L. (Nayar, 1963).

The purpose of control of aphids is not only to stop the progression of damage to plants by their increased proliferation but also to check the spread of viral and other microbial diseases. Aphids constitute one of the major carriers of viral disease in plants. (Carter, 1967). It is estimated that *Myzus persicae* alone is capable of transmitting as many as 90 out of 300 known virus diseases of plants. (Raychudhuri, 1980). The chief mode of transmission of such virus to a long distances is necessarily is through the alate morphs of the insects. (Mukhopadhyay and Sarkar, 1988). The ability of alate aphids to spread the diseases to plants constitutes a serious problem in the control of aphids and thereby the control of diseases of plants. Further, the use of Organophosphorus insecticides like Chlorpyrifos ,Malathion, Parathion, etc are of concern because of their toxicity and further the induction of insecticide resistance in aphids by the process of Gene amplification (Devonshire and Field, 1991; Sloderbeck et al, 1991).

Thus, an increasing attempts for the control of aphids by the use of insect hormones and analogues, anti-juvenile compounds as well as pheromones are the recent trends at the management of aphid as well the transmission of plant diseases by them.

It is therefore imperative that the use of anti-juvenile compounds like Precocenes, Pheromones like 'Queen substances', and many more such compounds may as a whole be used in one of such overall strategy in the control of aphids.

GENERAL DISCUSSION

From the present study, it is revealed that, the aphid *Macrosiphum rosaeiformis* is a highly polymorphic species, exhibiting upto six polymorph forms; occurring under specific time of the annual seasonal cycle. The presence of such multiple forms apparently signifies an effective evolutionary survival strategy of the species, in terms of its mode of life cycle. (Dixon, 1985). The plasticity of the individuals of a species of aphid to undertake a specific pathway out of several; towards a particular morph, is of a great asset in the ability of a species to survive any form of environmental contingency. (Ghosh and Roychoudhury, 1986-87)

The present study reveals the involvement of the environmental factor mainly,Photoperiod and temperature in the regulation of the differentiation of polymorphism. It is observed that under summer long day conditions, the chief morphs that are observed are the alate and apterous virginoparae, these morphs proliferate entirely by means of parthenogenetic viviparity, The differentiation of alate and apterous under long day condition is not fully understood, but from the literature on works in many species of aphid, it is revealed that interaction with individuals of the species brought about by crowding, a change in the nutritional status of the host plant due to overpopulation as well as due to seasonal changes in photoperiod, temperature etc are involved via mediation through the endocrine system (Lees, [illegible] 1980; Hardie and Lees,1985).It is believed that juvenile hormone, involved in metamorphosis is also responsible for the control of alary dimorphism. The difficulty however, in obtaining direct evidence on the involvement of the corpora allata is due to the size and delicacy of these insects, which have, however, been eased after the discovery of the anti-allatin 'Precocenes'. This compound, with its property of selective action on the corpora allata leading to degeneration of this gland, thus, acts as a chemical allatactomy. (Bowers,1985)

Juvenile hormone (JH) is believed to be of alary inhibitory in nature. Elimination of this affect by a diminishing titer of juvenile hormone is held responsible for the induction of alates. The alate and the apterous early nymphs appear identical and cannot be differentiated phenotypically. It is only during the early third instar stage that their wing buds can distinguish the alates from the apterous.

When, Juvenile hormone-III is topically applied at a dose of 1μgl/0.1μl/individual to early pre-alate instars of the aphid *Macrosiphum rosaeiformis* (Das), the individuals that develops into adults shows various degree of morphological aberrations. These individuals resemble the apterous forms to a considerable extent in lacking the alate features. The wings are considerably reduced and in some, the ptherothoracic segment is ill developed, lacking the usual sclerotisation as in case of the alate forms. This is corroborative with the observation in the aphid *Aphis nerii,* with the treatment of JH analogue, it was suggested that the treatment of JH analogue may inhibit the growth of wing pads by obliterating division of cells, or influence wing growth and expansion by cellular differentiation and activity (Srivastava and Chhibber, 1980). These features are more of a nature of juvenile instars rather than the apterous adult. Apart from these effects, some percentages of fifth instar supernumerary forms are also produced. The production of such effects by the application of juvenile hormone necessarily indicates the involvement of Juvenile Hormone in the regulation of alates-apterous forms. The failure to achieve total apterisation does not in anyway displace the involvement of juvenile hormone and the cause could be due to the existence of very discrete JH-sensitive periods during the course of an individual's development. Such sensitive period as observed in the aphid *Megoura* (Lees, A.D, 1980) and in case of *Aphis fabae* (Hardie, 1981b), as well as in case of other insects are short or may consists of a series of periods during which the tissues are insensitive to JH, and it is during such phases in the life cycle that the hormonal switching takes place. Thus, Juvenile hormone acts during discrete critical periods, i.e the juvenile hormone sensitive period. In general, the presence of juvenile hormone during a critical period, bring about a developmental switch, whereas its absence during a critical period, leads to changes in gene expression and a new developmental processes begins that launch the developing insect on a new developmental pathway. (Nijhout and Wheeler,1982; Nijhout, 1994). The cells of an insect become committed to a specific pathway of developmental fate during such period. Moreover, once the process of morphogenesis has already begun it is difficult to reverse the process, but can only be disrupted from its normal course, which thus, explains the development of degenerated structures in the form of morphological aberrations.

The action of juvenile hormone on morph differentiation may act either pre- or post natally on the developing embryos. Further, there is the influence of 'maternal effects', whereby, the determination of a morph is affected by the factors acting on the mother and the influence produced on the progeny. (Mousseau and Dingle, 1991). The mechanism is undoubtedly being mediated by the endocrine system *viz.* the corpora allata. (Lees, 1966; Hardie and Lees, 1985). The difficulty associated with obtaining direct evidences on the role of corpora allata/juvenile hormone in aphid polymorphism through classical allatectomy has been circumvented by the discovery of chemical allatectomy in the form of the compound Precocenes.

In *Macrosiphum rosaeiformis,* on treatment with both Precocene-I and Precocene-II, there wasn't an increase in the percentage of alate progeny. The few percentages of alate produced were not statistically significant in comparison to the control. This observation suggests that a diminished JH level being brought about by experimental corpora allata inactivation by Precocenes does not lead to induction of alate progeny. Thus, a lowered or diminishing JH level cannot be attributed solely for the differentiation of alate morphs under long day photoperiod. However, it cannot be conclusively inferred that Precocenes treatment doesn't lead to an increase in alate progeny. Since, the effects of Precocenes have not been fully evaluated in aphids, further, a difference in the response towards Precocenes has been observed on a number of aphid species, which suggests a species specific responses towards Precocenes.

The alate gynoparae of *Macrosiphum rosaeiformis* on the other hand, produced under condition of shortening photoperiod during the approaching winter months, is however, appears to be regulated by the level of juvenile hormone, brought about as a result of a shortening photoperiod. Correlation has been found between the activity of the corpora allata and photoperiodic conditions. The increased volume of the corpora allata along with the increased c/n ratio of the neurosecretory cells in the corpora allata of individuals placed under long day photoperiod suggests an enhanced activity of the gland during long day photoperiod. Based on these findings it can be safely inferred, that a higher percentage of apterous morphs during long day photoperiod can be ascribed to an elevated titer of juvenile hormone. And, under short day the corpora allata activity diminishes to a level whereby, the alate inhibiting effect of the Juvenile Hormone is suppressed, resulting in the increased percentages of alate virginopara or more appropriately the short day alate gynopara. This is further supported by the observation that individuals subjected under short day and treated with Juvenile Hormone-III brings about a significant reduction in the alate progeny in comparison to the untreated control. The alate and the apterous virginoparae both exhibit the same responses with

JH-III treatments. The involvement of juvenile hormone in alate gynopara production is thus demonstrated in *Macrosiphum rosaeiformis*.

The influence of photoperiod is mediated via the neurosecretory cells in the brain as demonstrated in *Megoura (*Steel and Lees, 1977; Steel,1978). In addition, the effect of photoperiod varies depending upon life cycle. In anholocyclic species like *Megoura,* shortening photoperiod directly produce ovipara, whereas, in holocyclic species like *Aphis fabae,* the gynopara is produced first, this subsequently give rise to the Ovipara. The role of the brain neurosecretory cells (Group-I) has been held responsible for the production of parthenogenetic virginoparous morphs. This has been observed on the aphid *Megoura*, where cauterisation of these cells, leads to an inhibition of the photoperiodic response. Individuals, which lacked these cells, produced only oviparous progeny even under long day photoperiod.(Steel and Lees, 1977) The mechanism by which, the photoperiodic response operates in the production of alate gynopara is not however well studied in aphids.

In *Macrosiphum rosaeiformis,* the gynopara, produced under the influence of short photoperiod are all alates, it is however not clear, as to how the neurosecretory cells producing the so called 'virginoparin' which is responsible for virginoparous development also lead to alate gynopara production.

In the present study, it is observed that externous application of juvenile hormone to short day treated virginoparous individuals can mimic the long day induction of apterous progeny morphs. This is consistent with the observation on *A. fabae*, where externous juvenile hormone application mimics the photoperiodic apterisation of the alate gynopara. (Hardie, 1980). Further, a change in the corpora allata activity is noticed in terms of *c/n* ratio and volume under short and long day condition, thus, supporting the involvement of Juvenile Hormone in apterous development under long photoperiodic conditions in *Macrosiphum rosaeiformis*. Such correlation on the corpora allata volume differences between long and short day photoperiodic condition have also been observed in the case of the aphid *Megoura* (Kats, 1980).

A change in the content and density of stainable neurosecretory material is observed in the group-I neurosecretory cells of the aphid *Macrosiphum rosaeiformis*. A higher Paraldehyde fuschin positive materials are observed in the group –I neurosecretory cells in case of short day than in case of the long day treated individuals. Such observation have been reported in case of the aphid *Megoura*, where the Paraldehyde positive granular contents in the Gr-I cells were more in case of short day than in the long day treated insects. (Kats, 1980). This clearly indicates a higher secretory activity of these cells under long day, and thus, indicates the role of these cells in the polymorph differentiation under photoperiodic conditions.

Aphids constitute one of the major pests infesting agricultural and horticultural plants. Apart from the physiological damage caused by their attack, these insects also transmit a number of viral and other microbial diseases. Control of aphids is mainly through the use of organic insecticides, although these insecticides are highly effective in controlling aphids, but due to the inherent problems associated with them like toxicity, persistence and environmental pollution, they are not encouraged for long term use. Aphids have the property of developing quick resistance against these insecticides by their ability of exhibiting the phenomenon of gene amplification. Therefore, any compounds that utilise the use of triggering an insects developmental hormone disturbance will be of immense potential as a control agent. It is in this regard, that an attempt was made to study the potential of these compounds in controlling these pests from proliferation as well as from inhibiting the transmission of microbial diseases.

Treatments of the alate early instars with the anti-allatin compounds Precocene- I and II were found to lead to the production of a wide range of morphological deformities in the adults. These individuals are incapable of flight. Application of 'Queen substance' also has been observed to inhibit the production of parturition, thereby, reducing the number of progeny born significantly. Therefore, the use of these compounds can no doubt be one of the strategies in the control of aphids pests from their rapid proliferation as well spread of diseases.

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BIODATA

Name : Sri Sudhanya Ray Hajong

Address: Present: Department of Zoology; North-Eastern Hill University,
Shillong-793 022.

Permanent : P.O Tura, Hawakhama, West Garo Hills,Meghalaya

Academic records:

Exam Qualified	Year	Univ/Board	Class/Div
Matric	1984	C.B.S.E	II Div
P.U. Sc	1986	N.E.H.U	II Div
B.Sc(Hons)	1989	N.E.H.U	II Div
M.Sc	1992	N.E.H.U	II Div

Research experience: 5 years.

NET/GATE: Qualified in CSIR-UGC NET, 1992, Roll No. 13133.

Fellowship awarded: i) Junior Research Fellowship (NET), 1994- 1996.
ii) Senior Research Fellowship (NET), 1996 –1999.

Seminars, Conferences, Workshops attended:

1) Regional Workshop on Advance Techniques in Electron Microscopy, RSIC, Shillong.
2) Regional Workshop on Electron Microscopy, RSIC, Shillong.
3) Regional Workshop on IR-UV-VIS Spectroscopy, RSIC, Shillong.
4) National Symposium on Advances in Environmerntal Biology, NEHU, Shillong.
5) Ninth Manipur Science Congress, Manipur University, Imphal.

Papers published, communicated, accepted.

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2. Hajong,S.R and Varman, A.R :Neurosecretory cells in the Brain of the Aphid *Macrosiphum rosaeiformis* (Homoptera: Aphididae).*'Annals of Entomology'*.. 1998. (Accepted)

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Occurrence of intermediate forms in the APHID *Macrosiphum rosaeformis* (Homoptera : Aphididae)

Aphids are polymorphic insects showing many forms, each morph arises under the influence of environmental factors like photoperiod and temperature[1,3]. These multiple forms have a survival value for the species, enabling them to escape the rigours of the environmental stresses like cold climatic conditions. The appearance of polymorphic forms in aphids are controlled by the endocrine system in conjunction with the environmental stimuli like photoperiod, temperature as well as crowding, nutrition *etc.* Each morph is distinct and arises only under the influence of given factors like day length coupled with high or low temperature. However, intermediate forms may develop as treak of nature and without any biological consequence.

Although, reports on intermediate forms in aphids are available, to our knowledge there is no information on the occurrence of any intermediate morphs of aphids in this part of India.[2,6] We have observed the occurrence of such forms in the rose aphid, *Macrosiphum rosaeformis*, during the course of our study on its biology and polymorphism in natural outdoor conditions on rose plants kept in screen house. We noticed some individuals during the months of January to March (1994-1997) with greatly distended abdomen and showing little or no movement. When these individuals were brought to the laboratory and dissected under the microscope, they were found to contain a few embryos, but when the hind tibiae was examined to accertain the morph of the individual it was noticed that it contained scent plaques which are found only in the oviparous females, indicating that it possesses both the characters of the viviparous female and the oviparous female morph. Further rearing of these individuals in the laboratory proved them to be sterile probably due to anatomical limitations. (Plate 1).

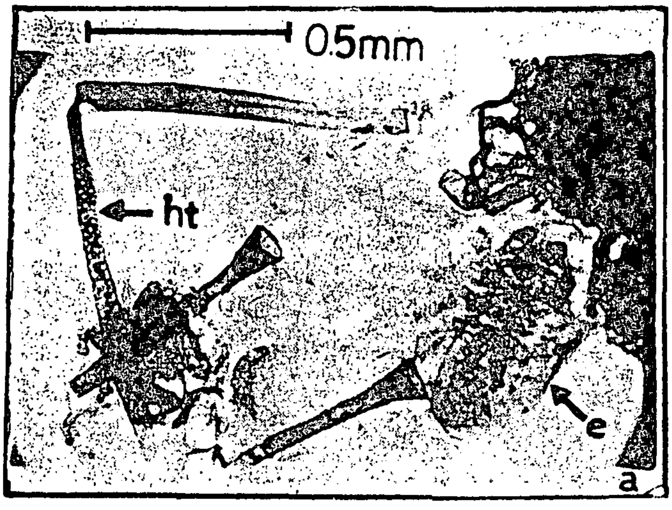

Plate I : Photograph showing the oviperous-viviparous intermorph. *(ht-hind tibiae showing scent plaques, e-embryo).*

Apart from the oviparous—viviparous intermorph few alate—apterous intermorphs could be observed during the months of November to March (Plate 2), these individuals had mainly crumpled and often fluid-filled swollen wings, their mesothoracic region was little sclerotization which is a characteristic of the apterous forms. These individuals, however, reproduce normally, producing young progency when reared in the laboratory.

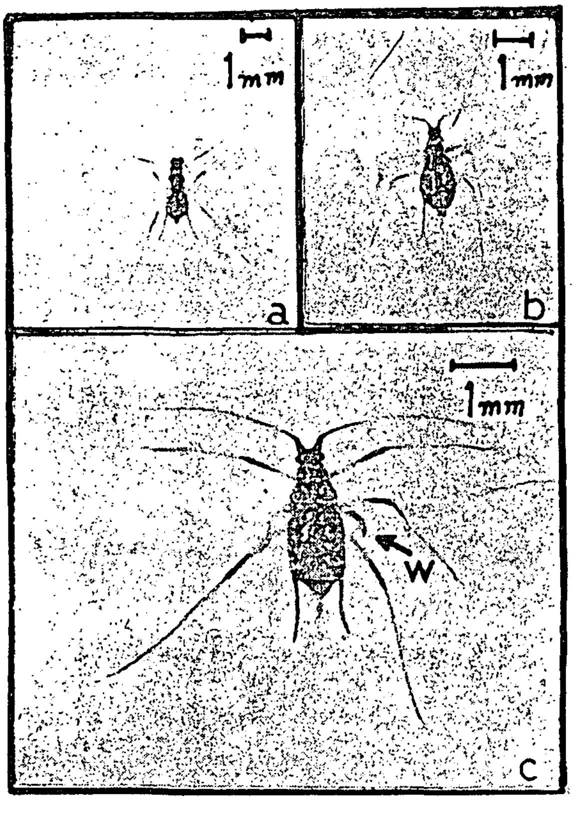

Plate II : Photograph showing the alate-apterous intermorph. (A normal alate vivipara B-intermorph showing crumpled wings, C-intermorph showing fluid-filled ill-developed wings).

The occurrence of these intermediates in the natural environment is of significance in understanding the physiological processes of aphid polymorphism as well as of importance to aphid systematics. It is established that environmental factors mainly photoperiod and temperature influence the endocrine system play significant role on the determination of polymorphic forms[5]. Studies on *Megoura* have shown that the median neurosecretory cells as well as juvenile hormone from corpora alata are responsible for the photoperiodic determination of the viviparous form under long-day condition.[8] Oviparous-viviparous intermediate forms are known to occur at the critical photoperiod or temperature and also at the switch-over points in the progeny sequence when the progeny maternal photoperiod is reversed,[4] thus, the apparent factor or influence is not maintained during the entire sensitive period of the developing embryo.

In case of the alate-apterous intermorph the mechanism is possibly same except that these intermorphs are actually the alate gynoparae which are the ovipara producers under reduced photoperiod and temperature as indicated by the period of its occurrence.

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S. R. HAJONG
A. R. VARMAN

Department of Zoology, North-Eastern Hill University
Shillong-793022.

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[1] A. F. G. Dixon, Biology of aphids, Edward Arnold (Publishers) Ltd. London, 1973.

[2] M. R. Ghosh and D. M. Raychaudhuri, Orient. Zool. 6 and 7, 1-11, 1986-87.

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