Effect of two juvenile hormone analogues on embryonic morphogenesis, histogenesis, endocrines and cuticulogenesis of *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae)

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Abstract. The effect of topical application of juvenile hormone analogues farnesyl methyl ether and kinoprene (ZR 777) at different doses to eggs immediately after laying, germ band formation and blastokinesis, produced different types of abnormal embryos with varying degrees of derangement of development, most of them ultimately resulting in failure to hatch. Some of the embryos were almost normal but failed to hatch even though they continued to develop inside the chorion and died two days later. On the whole, there was correlation between dose of the analogue applied and mortality rate. Kinoprene was much more effective than farnesyl methyl ether. With given dose, per cent embryonic mortality was more or less the same whether the analogues were applied just after oviposition or germ band formation, but was lesser when applied immediately after blastokinesis. The period just after germ band formation appeared to be most sensitive. Treatments affected the endocrine system. The neurosecretory index was higher in the treated embryos. Prothoracic glands and their nuclei showed considerable enlargement in treated embryos continuing development inside chorion even after their controls hatched. The corpus allatum was smaller in treated embryos and corpora cardiaca were filled with neurosecretory material. Cuticle development was abnormal after treatment.

Keywords. *Dysdercus cingulatus*; juvenile hormone analogues; kinoprene (ZR 777); farnesyl methyl ether; embryonic development; morphogenesis; histogenesis; endocrines; cuticulogenesis.

1. Introduction

Ever since Slama and Williams (1966) reported that ‘paper factor’ affected embryonic development in *Pyrrhocoris apterus*, instances of juvenile hormone (JH) analogues leading to abnormalities in embryonic development have been reported in insects belonging to different groups and with different analogues (Slama 1974). The effects vary depending upon species, time of application, dosage applied, and the hormone analogue used. Dipteran embryos appear to be more resistant than the others and heteropteran embryos are very sensitive (Matolin 1971; Wright and Spates 1972; Enslee and Riddiford 1977; Patterson and Schwarz 1979). Some of our findings on the effects of the JH analogues farnesyl methyl ether (FME) and kinoprene (ZR 777) topically applied to eggs of *Dysdercus cingulatus* at different stages of development, on morphogenesis and on the endocrines (Jacob and Prabhu 1979a, b), ontogeny of the endocrine organs of the normal insect and their probable involvement in embryonic moulting (Jacob and Prabhu 1985), effects of the analogues at cellular level constituting inhibition of mitotic division and pycnosis of nuclei arresting mitotic activities at various stages of embryonic development (Jacob and Prabhu 1986b), and the significance of the endocrines and exogenous JH in embryonic development in *D. cingulatus* (Jacob and Prabhu 1986a) have been briefly reported.
The present paper deals in detail with the effect of JH analogues FME and Kinoprene on morphogenesis, histogenesis endocrine organs and cuticulogenesis in this insect.

2. Materials and methods

Rearing methods, collection of eggs of required age, and techniques employed have already been described (Jalaja and Prabhu 1976; Jacob and Prabhu 1985). The JH analogues used were, FME purchased from Eco Control Inc., USA and Kinoprene (ZR 777) a gift from Dr G B Staal of Zoecon Corporation, USA. The analogues were dissolved in acetone, and 1 µl containing the required dose were topically applied by means of a microliter syringe; to eggs (i) at oviposition; (ii) immediately after germ band formation and (iii) just after blastokinesis. Acetone treated eggs served as controls. Each group consisted of 25 eggs, and the experiments were replicated several times to get sufficient number of embryos for the study. Their development was followed, by fixing and processing them as described for normal embryos (Jacob and Prabhu 1985).

3. Results and discussion

3.1 Abnormal embryos

FME and kinoprene (ZR 777) applied to eggs of D. cingulatus at different stages and doses resulted in different types of abnormal embryos. Figures 1–6 are the photographs of their whole mount preparations and figures 7–13 give their histological picture. Some of the embryos are comparable to those reported in Schistocerca gregaria (Novak 1969); some are comparable to those in Pyrrhocoris apterus (Matolin 1971) and some others resemble those reported in Pyrrhocoris by Enslee and Riddiford (1977); some are new abnormalities not reported earlier in any species. These abnormalities and their appearance and histological picture in D. cingulatus are described.

3.1a Non-segmented embryo: The non-segmented embryo was the most severe abnormality and showed maximum derangement of development. In whole mounts it appeared as red spot at the posterior pole of the egg (figure 1). However, the structure of those embryos varied, on the basis of which these may be divided into two:

(i) Undifferentiated embryo: This type of embryo was made up of disorganised mass of cells without differentiation into tissues or organs. Disintegrating nuclei were also observed in them. Blastoderm cells appeared to show no mitosis and this embryo did not proceed to form germ band.

(ii) Non-segmented embryo with rudiments of appendages: This type of embryo was divisible into a small anterior part, the head, and a tapering part together constituting the thorax and the abdomen. The embryo was only half the size of the egg. There were two or three appendage rudiments; those faced the yolk and hence were not visible in whole mounts. Appendage rudiments were not further divisible. A small brain and 3 thoracic ganglia could be observed in this embryo. The abdominal
region was represented by a few scattered cells most of which were big having pycnotic nuclei (figure 7). Some of these embryos showed a germ band with developing anterior region while the blunt end of the germ band did not show any differentiation. Masses of cells seemed to sink into the yolk from the anterior part of the germ band. The amnion cells appeared more spherical. It appears that the embryo remained at the posterior pole of the egg without undergoing blastokinesis.

3.1b *Dwarf embryo:* Though this showed marked differentiation (figure 2), it did not hatch and was quite common. The body was divisible into head, thorax and abdomen. All the appendages were present. The embryo was usually seen in the posterior pole or at the middle region of the egg. The embryo got pigmented on fourth day, the pigmentation deepened on fifth day and red eye spots developed. Till the normal time of hatching of controls, this embryo showed movements inside the chorion but it failed to hatch. A well-formed nervous system occupied the major part of the body. The gut was highly reduced. Neither muscle fibres nor gonadal rudiments got differentiated. When the JH treated eggs were fixed on third day, some of them...
appeared with completed germ band but the germ band and the appendages were shorter compared to controls (figures 3 and 4). Masses of undifferentiated cells could be seen in the inner layer.

3.1c Embryo without dorsal closure: This appeared frequently after JH treatment. The size of the embryo varied. It had no dorsal ectoderm and the gut was in direct contact with the yolk. Body was divisible into head, thorax and abdomen. All body segments and appendages were present irrespective of the size of the embryo. Two

Figures 7–13.
types of embryos were identifiable. In the first group, there was dorsal ectoderm which was however, incomplete. So the central portion of thorax and abdomen remained open (figure 5). This abnormal embryo got pigmented except at the open area. It had red eye spots, feeble muscle fibres and differentiated gonadal rudiments; the embryos got pigmented on fourth day. In the second type, the abnormality was more severe. It showed no sign of development of a dorsal ectoderm. The dorsal side remained without pigmentation.

3.1d Embryo with sunken head: This type of embryo was observed only after treatment with kinoprene. The head was not visible on the surface. The appendages appeared to originate from the dorsal side of the body. A number of foldings of ectoderm were observed at the thoracic region. This embryo was smaller. It got pigmented and body movements were observed till the hatching time of controls. The brain was shifted to about half the way through the dorsal side of the body. The central nervous system was smaller. The dorsal ectoderm was transparent; the gut was reduced in size. Muscle fibres were present on the ventral side of abdominal segments. Genital rudiments could not be identified in this embryo. Masses of undifferentiated cells could be seen in the abdominal region (figure 8).

3.1e Embryo with short and stumpy appendages: This was rather rare among the unhatched eggs. In this, none of the body segments were affected, though normal number of segments were lacking in the appendages (figure 6) Pigmentation and size was normal. Nervous system did not consolidate. The gut was enlarged occupying the major part of the embryo.

3.1f Less pigmented embryo: Normally, the embryos had light yellow pigmentation on fourth day which changed to an orange red shade before hatching. However, some of the treated embryos remained colourless while in others the light yellow pigmentation remained till fifth day. The eye pigmentation also was reduced considerably. The nervous system got consolidated but in most of the embryos the
neurons had no compact arrangement. Muscle fibres were distinguishable but no gonads differentiated. Masses of disintegrating cells were seen in the abdomen.

3.1g *Embryo which completed development but failed to hatch:* The most common abnormality was that which resulted in failure of apparently normal embryo to hatch. Till the hatching time the embryo showed movements inside the chorion; it got pigmented normally but the movements stopped a few minutes after normal hatching time of controls. Histological studies however revealed disintegrating nuclei throughout. All systems got differentiated but many nuclei were pycnotic.

3.1h *Embryos which died while hatching:* These constituted the embryos which apparently not only completed development but also made attempts and struggled to come out of the chorion, but failed to hatch. The appendages were wrapped in a transparent membrane. There were fewer disintegrating nuclei in them.

3.1i *Embryo continuing development inside the chorion even after hatching time:* Among the unhatched eggs, some continued development further, though rarely. These embryos showed movements till two days after normal hatching time of controls. Pigmentation changed to red like those of a two day old first instar nymph. These embryos were enclosed in a transparent membrane inside the chorion. Nervous system got consolidated; mesoderm derivatives were present, and the size was also normal.

It may be seen that embryos with sunken head have not been reported elsewhere; embryos with lesser pigmentation has been reported only in *Thermobia* (Matolin and Rohdendorf 1972). We have not noted any twin embryo as reported in *Thermobia* by Rohdendorf and Sehnal (1973).

3.2 *Hatchability studies*

There was clear correlation between dose of the analogue applied and mortality of the embryos including all the abnormalities produced ultimately resulting in failure to hatch, when either of the analogues were applied. However, 0.25 and 0.5 μg FME had little effect. Generally, the undifferentiated mass of cells referred to as non-segmented embryos, was the most severe abnormality. Kinoprene was much more effective than FME. The former was over 100 times active, its LD₅₀ is 1/125 of the latter. With given dose, per cent embryonic mortality was more or less the same whether the analogues were applied immediately after oviposition or germ band formation, there being no difference in mortality. However, the effect was less when applied immediately after blastokinesis. Germ band stage was the most critical sensitive period. However, with regard to the type of abnormality produced, there was considerable difference. In this respect the present findings on *D. cingulatus* are different from those on *Hyalophora cecropia* (Riddiford 1971) where the effect was delayed due to the presence in the eggs of longer lasting stored material gene products.

Mitotic inhibition and nuclear pycnosis and degeneration occurring especially at germ band formation (Jacob and Prabhu 1986b) are the causative factors of abnormalities resulting in embryos made up of undifferentiated mass of cells. Where severity of treatment was less, germ band formation continued, but blastokinesis
failed to occur; where the effect was least, blastokinesis also continued but hatching was prevented. Most of the embryos died at this point but some which struggled to hatch, continued development for some time till they succumbed to death. Endocrines were also affected, especially prothoracic gland activity; so cuticulogenesis and moulting were disrupted. It is clear from the studies that though corpus allatum was inactive during embryonic development in *D. cingulatus* (Jacob and Prabhu 1985), exogenous JH affects embryonic development decisively.

### 3.3 Endocrine system

The data on the neurosecretory system is analysed and presented in table 1. It may be seen that neurosecretory index is lower in control insects whereas in treated ones resulting in abnormal development, the index is comparatively high, the greater the abnormality, the higher the neurosecretory index. Thus, the embryos which died while hatching, had almost the same index as in the controls whereas non-segmented as well as dwarf embryos (figure 9) had many times the index compared to that of the controls at the time of hatching. Neurosecretory cell volume and nuclear volume of embryos with drastic abnormalities, were very high, though in many other types of abnormal embryos their values were slightly less than in the controls. Prothoracic gland cells and their nuclei were considerably enlarged in treated embryos which continued development inside chorion even after normal hatching time (figure 11). In other abnormal embryos they were more or less comparable to controls (table 1). Corpus allatum (table 1) was comparatively smaller in treated embryos except in those which continued development inside chorion. This could be due to a negative feedback action of exogenous analogue applied. Though during normal development corpus allatum did not show much evidence of activity as indicated by increase of size (Jacob and Prabhu 1985) the present finding of feedback inhibition of exogenous JH analogues in treated embryos showed that they were active at least in 5 day embryos. Corpus allatum of the treated individual could not fully compensate for the excess titre by inhibiting its own activity and abnormality results from the resultant upset of hormonal milieu. Treatment after blastokinesis produced lesser effect evidently because by then corpus allatum developed and started its activity and its own shutting off of activity to a certain extent regulated the hormone titre. It may be noted that though juvenile hormones and ecdysteroids have been reported in eggs and embryos of insects (Hoffmann and Lagueux 1985) there is very little evidence that juvenile hormone is indeed synthesized in the embryo corpus allatum. The present finding of a feedback inhibition of allatum in treated embryos shows that the corpus allatum is active at least in 5 day old embryos though it was not possible to observe any size increase of corpus allatum in our earlier studies (Jacob and Prabhu 1985). Evidence of capability of embryos for JH synthesis has been obtained by Bergot *et al.* (1981) in *Manduca sexta* and the present findings localises this site at corpus allatum, in *D. cingulatus*. Unlike in the case of corpus allatum, it has been possible to demonstrate active synthesis by embryonic prothoracic glands, of ecdysteroids; our earlier findings (Jacob and Prabhu 1985) suggest activity of the glands in the embryo in *D. cingulatus*, and the present work shows exaggerated size increase of prothoracic gland cells and their nuclei after exogenous treatment of JH especially in embryos which continued development inside chorion even after hatching time, implicating greater activity of the prothoracic glands in these treated
Table 1. Changes in the neurosecretory cells, prothoracic gland cells and corpus allatum of 5 day old abnormal embryos resulting from JH treatment (Mean ± SD).

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>Neurosecretory cells</th>
<th>Prothoracic gland</th>
<th>Corpus allatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell volume (µm³)</td>
<td>Nuclear volume (µm²)</td>
<td>Cell volume (µm³)</td>
</tr>
<tr>
<td>Non segmented embryo</td>
<td>1554.09 ± 213.89</td>
<td>423.25 ± 52.42</td>
<td>Prothoracic gland not differentiated</td>
</tr>
<tr>
<td>(702.90 ± 28.19)</td>
<td>(301.01 ± 8.60)</td>
<td></td>
<td>Corpus allatum</td>
</tr>
<tr>
<td>Dwarf embryo</td>
<td>1660.12 ± 315.55</td>
<td>403.50 ± 157.65</td>
<td>49.64 ± 1.55</td>
</tr>
<tr>
<td>(698.90 ± 67.62)</td>
<td>(291.11 ± 9.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos without dorsal closure</td>
<td>599.46 ± 12.02</td>
<td>178.08 ± 23.59</td>
<td>66.63 ± 2.58</td>
</tr>
<tr>
<td>(159.44 ± 6.28)</td>
<td>(60.03 ± 3.98)</td>
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</tr>
<tr>
<td>Embryos with sunken head</td>
<td>143.75 ± 7.33</td>
<td>32.64 ± 1.69</td>
<td>71.89 ± 1.04</td>
</tr>
<tr>
<td>(93.28 ± 4.11)</td>
<td>(22.54 ± 4.47)</td>
<td></td>
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<tr>
<td>Embryos with short and stumpy appendages</td>
<td>130.25 ± 24.33</td>
<td>19.72 ± 3.28</td>
<td>78.97 ± 1.35</td>
</tr>
<tr>
<td>(103.24 ± 14.09)</td>
<td>(16.01 ± 2.27)</td>
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<tr>
<td>Embryos with less pigmentation</td>
<td>324.87 ± 10.80</td>
<td>83.54 ± 3.03</td>
<td>65.44 ± 1.23</td>
</tr>
<tr>
<td>(117.84 ± 8.14)</td>
<td>(41.76 ± 4.46)</td>
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<tr>
<td>Embryos which completed development but failed to hatch</td>
<td>169.40 ± 13.09</td>
<td>25.17 ± 5.91</td>
<td>47.71 ± 1.35</td>
</tr>
<tr>
<td>(102.51 ± 19.61)</td>
<td>(14.13 ± 1.52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos which died while hatching</td>
<td>164.31 ± 10.09</td>
<td>20.11 ± 1.76</td>
<td>46.45 ± 0.70</td>
</tr>
<tr>
<td>(105.78 ± 12.80)</td>
<td>(17.67 ± 1.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos which continued development inside the chorion</td>
<td>279.39 ± 81.35</td>
<td>57.67 ± 8.19</td>
<td>381.70 ± 3.52</td>
</tr>
<tr>
<td>(153.75 ± 22.42)</td>
<td>(49.38 ± 5.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>343.70 ± 29.18</td>
<td>63.18 ± 2.61</td>
<td>73.62 ± 1.55</td>
</tr>
<tr>
<td>(173.51 ± 10.91)</td>
<td>(31.61 ± 2.17)</td>
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Values of pear shaped neurosecretory cells are given in parentheses. Those outside are of spherical cells.
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embryos. On the whole the embryos which continued development inside the chorion are the least affected ones. Their greater prothoracic gland activity was indicative of some problem with moulting. The most affected ones are the non-segmented embryos, dwarf embryos and those with dorsal closure, as it was before corpus allatum developed and hence could not regulate the hormone titre by regulating its own production.

Additional noteworthy features of the changes in the endocrine system after treatment are, that the corpora allata, corpora cardiaca and the prothoracic glands were not differentiated in the non-segmented embryos. The corpora cardiaca were filled with secretory material in almost all treated, abnormal embryos (figure 10); the corpora allata showed no cyclic activity in which respect it was comparable to that of the controls; however, they were smaller in size in treated individuals. The prothoracic glands had considerable pycnotic nuclei, fewer cells showing cyclic activity (figure 12). Exceptions were those embryos which continued development inside the chorion; fewer nuclei were pycnotic in them and their nuclei had increased in size considerably.

3.4 Cuticulogenesis

No cuticle was found in non-segmented embryos. However, in the dwarf embryos there were 3 cuticles covering the appendages (figure 13) whereas only one cuticle covered the dorsal and ventral aspects of the body. In the embryos without dorsal closure, two embryonic cuticles were observed around the appendages and ventral aspects of the body. These appeared to represent the first and the second cuticles as they were very thin, the thicker third cuticle attached to the epidermis. Only one moult has taken place in these abnormal embryos. The embryos with sunken head had only one cuticle almost throughout the body. At the head region however, thick cuticle was evident. Around the head and appendages of the embryo with short and stumpy appendages, two cuticles were clear. They represented the second and third cuticles, as the cuticle attached to the epidermis was thicker. Only one cuticle was seen in the ventral and dorsal aspects of the body. Less pigmented embryos had only one cuticle covering the body. This cuticle got detached at different regions of the dorsal and ventral sides but no new cuticle was seen beneath the detached cuticle. In these embryos only the first cuticle was initiated and even that was not complete. In the embryos which completed development but failed to hatch, those which died while hatching and those which continued development inside the chorion even after hatching, had a transparent membrane covering the embryos when the chorion is removed. In histological preparations this membrane could be identified as the composite of two cuticles which are closely packed together. They got detached from the embryo. In addition, there was a thick, third cuticle attached to the epidermis. Thus these embryos had all the 3 cuticles characteristic of the normal embryo. Two abortive moults occurred in these embryos, though they failed to hatch ultimately. Hatching was prevented due to the presence of this membrane constituting the composite first and second cuticles. This situation is reminiscent of the condition in Spodoptera littoralis where excess juvenoids entail blockage of metamorphosis, the treated larvae continuing to grow until the juvenoids disappear, never moulting till they perish even after 30 days (Gelbic and Sehnal 1986). Hyperactivity of the prothoracic glands as indicated by the larger size of the cells and the nuclei observed in
the present study is an attempt on the part of the prothoracic glands to circumvent the excess JH analogue present in the embryo. It may be noted that coinciding with embryonic molts, balanced high peaks of ecdysone and juvenile hormone have been reported (Imboden et al 1978) which are necessary for embryonic cuticulogenesis and molting. Exogenous juvenile hormones are also known to inhibit ecdysterone synthesis not only in insects like Blattella germanica (Masner et al 1975) but in embryos as well, as in Rhodnius prolixus (Patterson and Schwarz 1979). In Oncopeltus fasciatus embryos the source of intrinsic ecdysteroid production is thought to be oenocytes (Dorn and Romer 1976) whereas in D. cingulatus embryos the present study confirm our earlier suggestion that prothoracic glands appear to be involved in ecdysteroid synthesis (Jacob and Prabhu 1985).

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References

Dorn A and Romer F 1976 Structure and function of prothoracic glands and oenocytes in embryos and last larval instars of Oncopeltus fasciatus (Insecta: Heteroptera); Cell Tissue Res. 171 331–350
Gelbic I and Sehnal F 1986 Failure of Spodoptera littoralis (Lepidoptera) to produce superlarvae; Acta Entomol. Bohemoslov. 83 161–170
Jacob M and Prabhu V K K 1979a Morphogenetic abnormalities caused by treatment of eggs of Dysdercus cingulatus with JH analogues; Curr. Sci. 48 505–507
Jacob M and Prabhu V K K 1979b Effects of two juvenile hormone analogues on embryonic neuroendocrine system of Dysdercus cingulatus (Heteroptera: Pyrrhocoridae); Entomonom 4 205–208
Jacob M and Prabhu V K K 1986a Dysdercus cingulatus Fabr. (Pyrrhocoridae: Heteroptera); What the endocrines and exogenous juvenile hormone analogues do during its embryonic development; in Recent advances in insect physiology, morphology and ecology (ed.) S C Pathak (New Delhi: Today and Tomorrow's Printers and Publishers) pp 103–134
Jacob M and Prabhu V K K 1986b Influence of juvenile hormone analogues on mitotic division in the embryos of Dysdercus cingulatus Fabr. (Heteroptera: Pyrrhocoridae); Proceedings of III Oriental Entomology Symposium, Part II (Kariavattom: Association for Advancement of Entomology) pp 1–6
Julaja M and Prabhu V K K 1976 Effects of chemosterilants apholate and metepa on the ovaries of the red cotton bug Dysdercus cingulatus Fabr. (Heteroptera: Pyrrhocoridae); Entomonom 1 43–53
Masner P, Hangartner W and Suchy M 1975 Reduced titres of ecdysone following juvenile hormone treatment in the German cockroach Blattella germanica; J. Insect Physiol. 21 1755–1762
Matolin S 1971 Effets d'un analogue de l'hormone juvenile sur les embryons de trois orders d'insectes; Arch. Zool. Exp. Gen. 112 505–509
Matolin S and Rohdendorf E B 1972 Effect of farnesyl methyl ether vapours on the embryogenesis of Lepismodes inquilinus (*Thermobia domestica*) (Thysanura); *Acta Entomol. Bohemoslov.* 69 1–6
Novak V J A 1969 Morphogenetic analysis of the effects of juvenile hormone analogues and other morphogenetically active substances on embryos of Schistocerca gregaria (Forskal); *Embryol. Exp. Morphol.* 21 1–21
Patterson J W and Schwarz M 1979 The activity of juvenile hormone mimics for the eggs of Rhodnius prolixus; *J. Insect. Physiol.* 25 399–404
Rohdendorf E B and Sehnal F 1973 Inhibition of reproduction and embryogenesis in the fire brat, *Thermobia domestica* by juvenile hormone analogues; *J. Insect. Physiol.* 19 37–56
Slama K 1974 Physiological and biochemical effects of juvenoids; in *Insect hormones and bioanalogues* (eds) K Slama, M Romanuk and F Sorm (Wien, New York: Springer-Verlag) p 477
Wright J E and Spates G E 1972 Laboratory evaluation of compounds to determine juvenile hormone activity against the stable fly; *J. Econ. Entomol.* 65 1346–1349