FLEA EGGS: TARGET OF THE NEW IGR ON-ANIMAL TREATMENTS

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Abstract—Currently 3 insect growth regulators either are or soon will be labelled as on-animal treatments to control fleas on pets and livestock. These chemicals, fenoxycarb, methoprene and pyriproxyfen are juvenile hormone mimics that disrupt embryonic and post-embryonic development when present during certain critical phases of cellular transformation. When applied to the host animal, these compounds either act internally in the adult flea to prevent egg development or they act systemically in the flea eggs to prevent development of the subsequent adult stage.

Recently completed studies showed that pyriproxyfen has more than one effect on adult fleas. Adults exposed to pyriproxyfen in glass vials prior to feeding on a cat, deposited little or no yolk in their eggs over the next three days. Yolk was deposited in eggs produced during days 3 and 4 but the embryos failed to grow because no organized cleavage center developed. Thus, normal egg production was disrupted for at least 4 days after pyriproxyfen exposure. Similar studies with methoprene treated fleas showed no effect on yolk deposition. However, eggs laid during the first 3 days after fleas exposed to methoprene were placed on a cat, failed to survive because the larvae either died as fully formed embryos or died soon after hatching.

In addition to IGR uptake during ovarian development, flea eggs are also affected by these insecticides. Exposure of eggs to treated pet fur for just 1 minute can disrupt embryonic or larval development. Microscopic examination of eggs revealed that this rapid effect is due to the highly permeable chorion or shell of these eggs. Flea eggs were found to have a unique chorion consisting of only a monolayer of gelatinous material overlying the vitelline membrane. This non-sclerotized chorion evidently allows rapid penetration of insecticides before the egg falls from the host.

INTRODUCTION

Of the various insects classified as household pests, fleas are among the most vulnerable to control with insect growth regulators (IGRs). Both the egg and larval stages are extremely sensitive to IGRs, thus, they can be controlled before the pest stage adult flea develops by spraying the house and yard periodically during the spring and summer months. At least 3 IGRs which mimic the action of juvenile hormone (JH) are or soon will be available for flea control throughout the world, fenoxycarb, methoprene and pyriproxyfen (Fig. 1). Fenoxycarb and pyriproxyfen are relatively new, photostable anologs of JH. This report will consider the effect of pyriproxyfen and methoprene on cat fleas, the most common pest flea of dogs and cats.

In the United States, one of the significant new developments in flea control involves the use of JH mimics as pet sprays formulated for direct application to the animal. This approach exploits the host- parasite relationship by placing the IGR on the fur of the dog or cat where adult cat fleas live and reproduce. Methoprene and pyriproxyfen have recently been reported to affect reproduction by being absorbed through the cuticle of adult fleas (Palma, *et al.*, 1993), while a number of investigators have shown that JH mimics can also penetrate the shells of newly laid eggs and prevent the development of the cat flea embryo (Olsen, 1985; Marchiondo *et al.*, 1990; Young and Donahue, 1992; Meola *et al.*, 1993). Thus, these IGRs have more than one means of affecting the reproduction of fleas.

Two topics will be addressed at this time: 1) how pyriproxyfen and methoprene affect egg production and embryogenesis; 2) why the flea egg is so permeable to JH mimics.

MATERIALS AND METHODS

Adult Treatment

Fleas used in this study were taken from a colony of cat fleas, *Ctenocephalides felis* (Bouch)), maintained at Texas A&M University. For the adult tests, 7 to 14 day-old fleas of both sexes were exposed to pyriproxyfen or methoprene residues in glass vials. Technical grade dilutions of the

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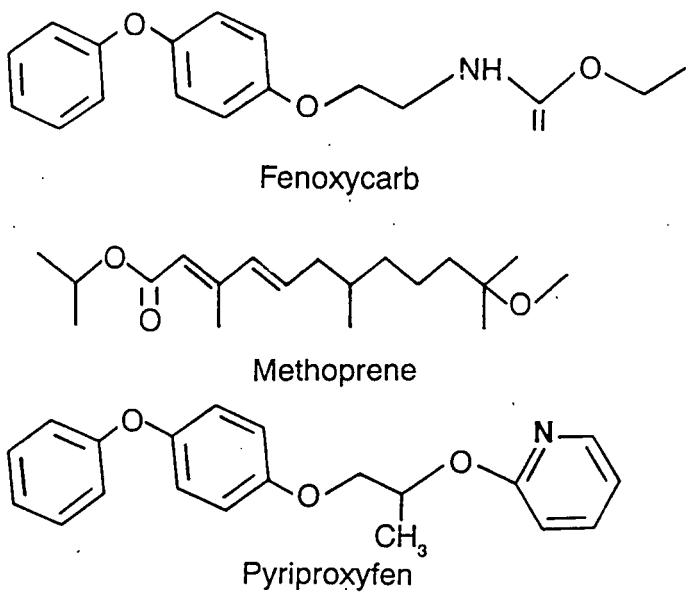

Figure 1. Comparison of the chemical structure of three IGR mimics of JH suitable for flea control.

IGRs were prepared in acetone and added to the vials in quantities of 10 mg/vial. The vials were then rolled on a flat surface to disperse the chemicals evenly over the inner surface at a rate of 0.25 g/cm. Control vials were treated the same using only acetone. The vials were dried 3 hrs in a fume hood to remove the acetone, then 50 adult fleas were placed in each vial for 24 hrs at 27°C and 80% RH prior to being placed on a cat. Eggs from these fleas were collected at intervals and held at the same temperature and humidity used for the adults. Control fleas from the acetone treated vials were placed on a second cat. Eggs from these fleas were collected at the same time interval as those from the treated fleas.

Histological Preparation

Eggs were prepared for light microscopy by fixation in 4% glutaraldehyde in Gomeri's 0.1 M phosphate buffer pH 7.2 (Humason, 1962). The eggs were then freeze dried by placing them in porous plastic baskets (American Optical, Buffalo, NY), immersing them 2 minutes in liquid nitrogen, followed by 12 hrs lyophilization in a Speed Vac (Savanty, Hicksville, NY). The lyophilized eggs were placed in 100% methanol and then in 0.5% eosin in methanol for 3 hrs to enhance their visibility for subsequent preparation. The eggs were then rinsed in 2 changes of toluene prior to embedding in Paraplast (Sherwood Medical Laboratories, St. Louis, MO). Seven micron sections were stained with a modification of Mallory-Heidenhain stain (Cason, 1950).

Scanning Electron Microscopy Preparation

Flea eggs were fixed and freeze-dried as for histology mounted on SEM specimen stubs with double sticky tape and sputter-coated with gold and viewed at 10 kv with a Cambridge 200 scanning electron microscope.

RESULTS

Cat flea eggs are oval in shape and measure about 0.5 mm in length and 0.3 mm wide. When viewed with a dissecting microscope, they resemble white jelly beans (Fig. 2).

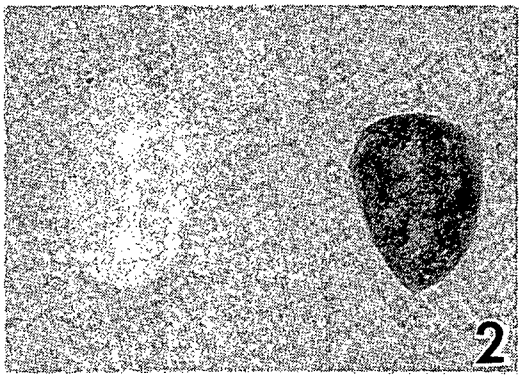

Figure 2.Comparison of a typical egg 34 hr after oviposition by a flea treated with pyriproxyfen compared with a control egg of the same age. Note the blackened, collapsed chorion of the treated egg. 54X.

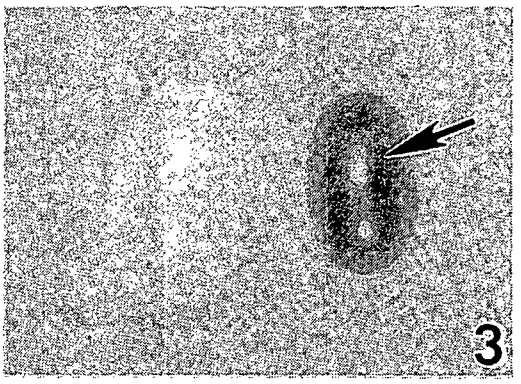

Figure 3.Comparison of a typical egg 43 hr after oviposition by a flea exposed to methoprene compared with a control egg of the same age. Note the darkened chorion of the methoprene treated egg contains a fully developed dead larva (arrow). 60x.

Pyriproxyfen Treatment

One of the first effects noted in pyriproxyfen-treated fleas was premature egg deposition. Fleas began depositing eggs 24 hrs after being placed on a cat, at least 6 hr earlier than normal. These eggs as well as those oviposited at 30 and 34 hr lacked yolk, and darkened and collapsed soon after being laid (Fig. 2). Therefore, premature oviposition and suppression of yolk deposition were two effects of pyriproxyfen on adult fleas. Pyriproxyfen-treated fleas produced abnormal eggs for 56 hrs.

The effects of pyriproxyfen diminished with time and after 46 hrs, the fleas began to deposit eggs with small amounts of yolk and by 70 hrs after the fleas were placed on a cat, they began to produce normal eggs. Eggs of pyriproxyfen-treated females collected at 46 hrs contained a distinct periplasm cap and reticular fibres associated with cleavage nuclei were present throughout the yolk but no organized cleavage centre had developed (Fig. 4). When these treated eggs were compared with control eggs showing cephalic lobe development within 14 hrs (Fig. 5), it was evident that pyriproxyfen prevented cellular differentiation. Eggs collected after 50 hrs had some cellular development but no blastoderm had formed.

Methoprene Treatment

Unlike pyriproxyfen which caused premature oviposition, methoprene- treated fleas did not begin laying eggs until 43 hrs after being placed on a cat, whereas the control fleas began oviposition at 33 hrs. In contrast to the pyriproxyfen-treated fleas, eggs laid by methoprene- treated adults showed no outward evidence of an IGR effect. However, most of the eggs collected at 43 and 47 hrs did not

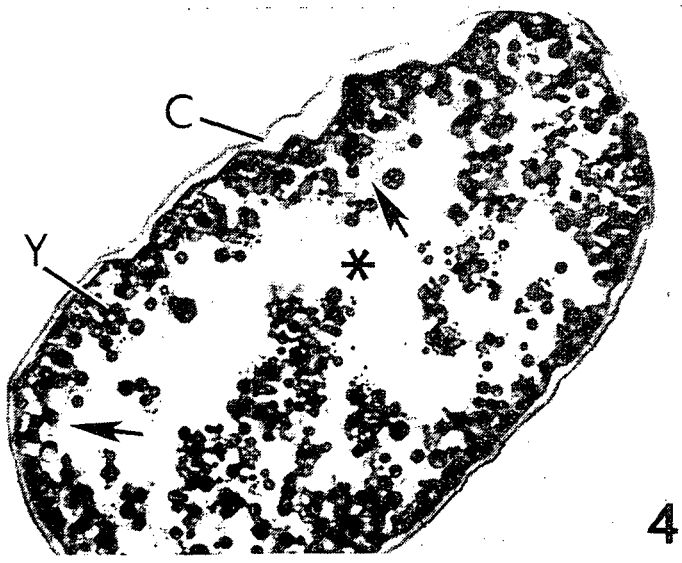

Figure 4.Histological section through a pyriproxyfen-treated egg 46 hr after oviposition. Arrows indicate reticular fibres and absence of periplasmic cap, thus no blastoderm has developed. Note the paucity of yolk in the central region of the egg (asterisk). C, chorion; Y, yolk. 280x.

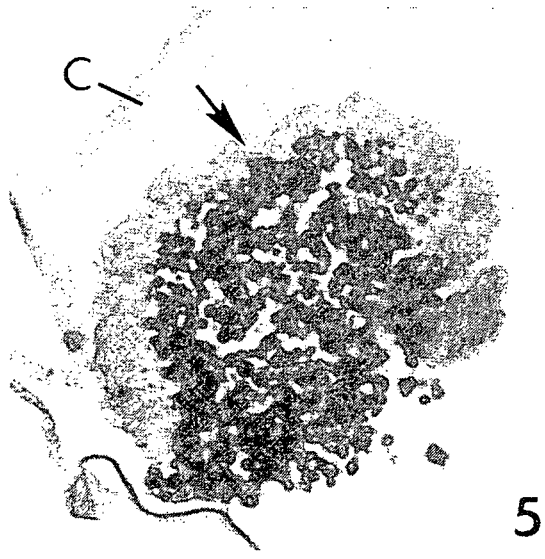

Figure 5.Section through a 12 hr untreated egg showing a well developed blastoderm (arrow) and an abundance of yolk throughout the ooplasm of the egg. C, chorion. 250x.

hatch, darkened and contained a fully developed dead larva (Fig. 3). Since larval anal hairs could be seen through the chorion, it was apparent that the larvae were fully developed. The larvae that hatched died within 1 to 2 hrs after hatching even though they were offered food and maintained in a moist environment. Eggs from both methoprene-treated and control fleas appeared to contain equivalent quantities of yolk, indicating that methoprene did not affect yolk deposition as did pyriproxyfen.

Histological sections of eggs from methoprene-treated fleas collected at 43 and 47 hr after fleas were placed on a cat, showed normal embryonic development compared with control eggs of the same age (Figs. 6 and 7). Eggs laid after 52 and 64 hrs hatched and developed through the first instar indicating that methoprene, like pyriproxyfen, gradually loses its effectiveness after fleas have been removed from the treated surface.

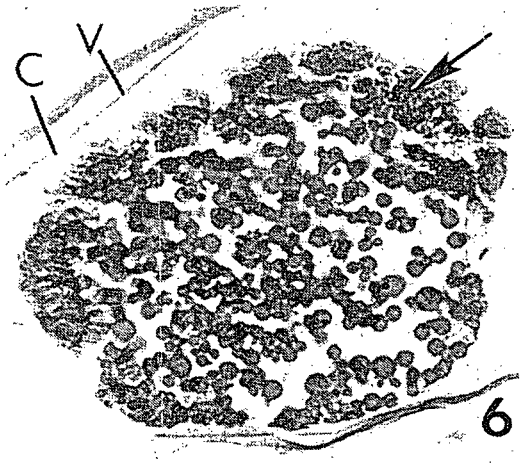

Figure 6.Section through an egg oviposited by a methoprene-treated flea 43 hr after being placed on a cat demonstrating normal embryonic development. The blastoderm (arrow) has formed and the ooplasm of the egg contains an abundance of yolk granules. C, chorion; V, vitelline membrane.

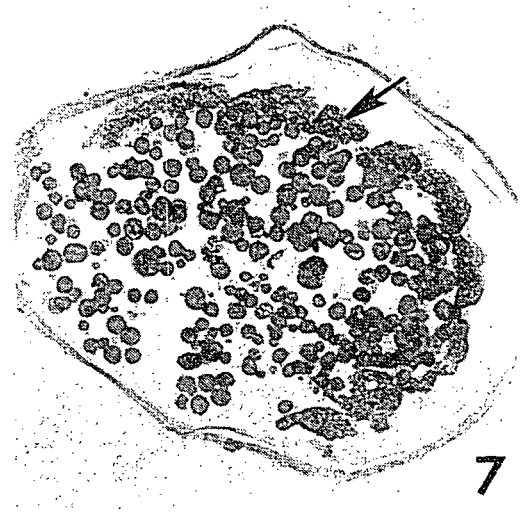

Figure 7.An egg 4 hr after oviposition by an untreated flea showing a similar stage of embryogenesis to that of the egg from a flea exposed to methoprene. Blastoderm, arrow. 225x.

Morphology of the Egg Shell

Scanning electron microscopy showed that the shell or chorion of the flea egg has a smooth surface lacking the distinctive patterns of most insects eggs. The chorion consists only of a single layer of cuticle overlayed by a covering of extra-chorionic material called spumaline (Hinton, 1981), which is produced by the collateral glands (Fig. 8). The fractured surface of the chorion reveals that it is non-lamellar, having an amorphous gel-like structure only 1 μm in thickness (Fig. 9). The chorion overlays the vitelline membrane and at higher magnification both the inner and outer surface of the chorion is composed of spheroid- shaped proturbances (Fig. 9).

DISCUSSION

The results of this study indicate that exposure of cat fleas to IGR- treated pet fur is likely to have a number of effects on both adults and eggs. When adult fleas were exposed to pyriproxyfen and then fed on a cat, the first eggs produced were aborted before yolk deposition was completed. Even when yolk was deposited in the eggs of older fleas, the embryos failed to complete blastoderm formation. Thus fleas continuously exposed to pyriproxyfen would probably fail to produce viable

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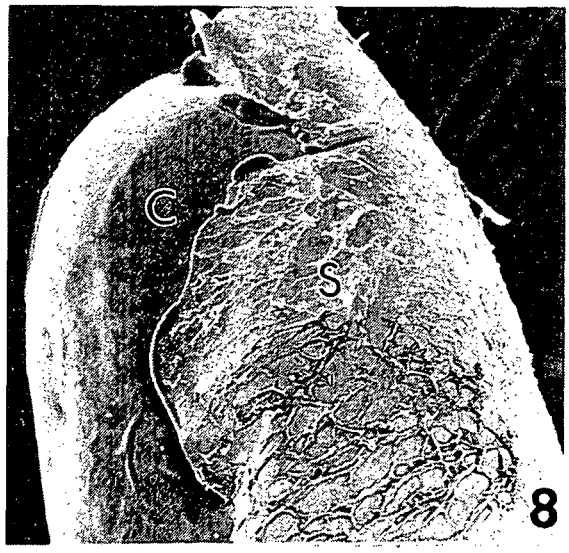

Figure 8.Scanning electron micrograph of a whole egg that reveals the spumaline membrane (S) covering the unsculptured chorion (C). 275x.

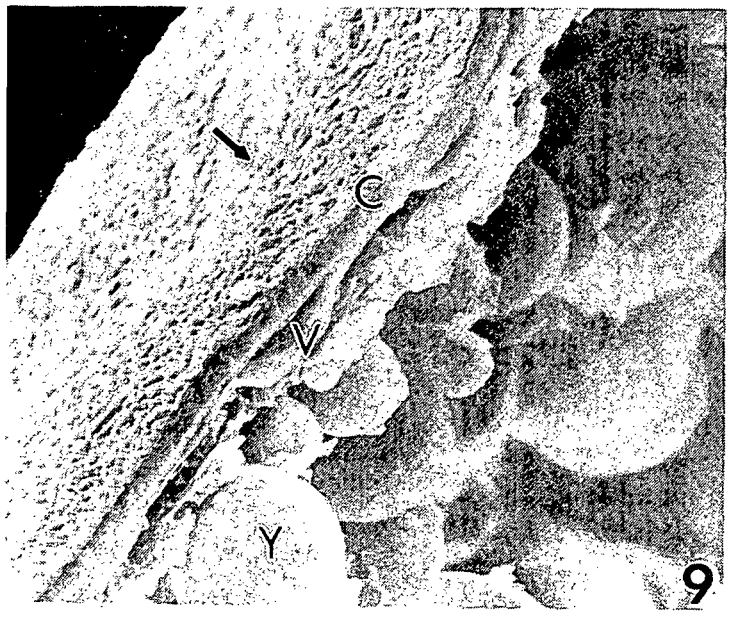

Figure 9.Higher magnification of an untreated, fractured flea egg that reveals the surface of the chorion (C) covered with minute spheres (arrow). The fractured surface through the width of the chorion is smooth and non-lamellate. V, vitelline membrane; Y, yolk. 3000x.

eggs. In contrast, fleas exposed to methoprene produced what appeared to be normal eggs. However, larvae developing in the eggs either died before or 1-2 hr after hatching. Interestingly, embryos of these eggs completed both the blastoderm formation and blastokinesis, stages of embryogenesis normally disrupted by JH treatment in other insects (Riddiford, 1992).

While adult fleas must remain in contact with JH-treated substrates to be continuously affected by IGRs, eggs can be affected by very short exposure intervals. As shown by Marchiondo *et al.* (1990), eggs exposed to fenoxycarb for 60 seconds are incapable of embryonation. Conversely, pyriproxyfen exposure can cause both embryonic and post embryonic effects. Meola *et al.* (1993) reported that these latent effects are transmitted through the embryo to the newly developed larva which then dies either before or shortly after hatching.

Permeability of the flea egg to JH mimics is apparently due to the presence of a thin, single-layered gelatinous chorion. This porous shell may be an advantage as a storage media for air when the egg is immersed in water, but this porosity apparently creates a surface that is readily penetrated by lipophilic agents such as JH mimics, that are able to penetrate the egg within minutes after exposure. This flaw in biological engineering would appear to make the egg especially vulnerable to IGR residues applied to host animal fur where the eggs are laid.

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