PHYSIOLOGICAL EFFECTS OF THE JUVENOID PYRIPROXYFEN ON ADULTS, EGGS, AND LARVAE OF THE CAT FLEA

R. MEOLA[1], S. READY[1] AND S. MEOLA[2]
[1]Department of Entomology, Texas A&M University, College Station, TX 77843-2475, USA
[2]USDA/ARS Food Animal Protection Research Laboratory, Route 1, Box 810, College Station, TX 77845, USA

Abstract—Adult cat fleas often survive several weeks without feeding. However, when newly emerged adults were maintained on filter paper treated with pyriproxyfen at 1.1 µg/cm, they lived only 8 days, compared with low mortality in the controls. Dying fleas examined histologically showed evidence of fat body depletion and midgut distention with air suggesting that death might be due to starvation. This finding was consistent with the hypothesis that unfed fleas undergo an adult diapause caused by failure of the corpora allata to release juvenile hormone (JH). However, subsequent experiments did not support this hypothesis. Exposure of unfed fleas to residues of JH III did not cause mortality, and fed fleas maintained on pyriproxyfen-treated dog hair died at the same rate as unfed fleas maintained on treated filter paper.

Studies involving flea eggs suggested that pyriproxyfen is less effective as an ovicide than fenoxycarb. Marchiondo *et al.* (1990) showed that newly laid eggs exposed to fenoxycarb treated filter paper for 60 seconds failed to hatch. Pyriproxyfen exposure of newly laid (1-4 hour-old) eggs to the same dose rate did not prevent hatching. However, 10 minute exposure of eggs to pyriproxyfen killed 50% of the fleas that developed to the larval stage. Thus pyriproxyfen had an unusual latent effect in which short-term exposure of flea eggs early in embryogenesis was often lethal to larvae that hatched from the egg 3 days later. In contrast, longer-term (2 hour) exposure of eggs to pyriproxyfen caused embryocidal effects.

INTRODUCTION

Pyriproxyfen is a new insect growth regulator that mimics the action of JH. It is similar in structure to fenoxycarb, sharing the 4-phenoxyphenoxy group, but is quite different chemically from methoprene and JH III (Fig. 1).

In fleas, the mode of action of pyriproxyfen is similar to methoprene. Pyriproxyfen accumulates in developing larvae where it apparently acts like JH to maintain genes that direct production of larval cuticle. The presence of pyriproxyfen during metamorphosis prevents fleas from successfully completing either the larval-pupal or pupal-adult moult causing the flea to transform into a larval-pupal or a pupal-adult intermediate that can neither function nor reproduce.

Recently, Palma *et al.* (1993) showed that pyriproxyfen also may be absorbed into adult cat fleas where it causes premature deposition of underdeveloped eggs or has embryocidal effects. These newly discovered modes of action suggested that pyriproxyfen might have other deleterious effects on fleas. The results of the present study revealed two additional modes of action:

1. Pyriproxyfen toxicity to adult fleas
2. Latent effects on larval fleas caused by short-term exposure of newly laid eggs

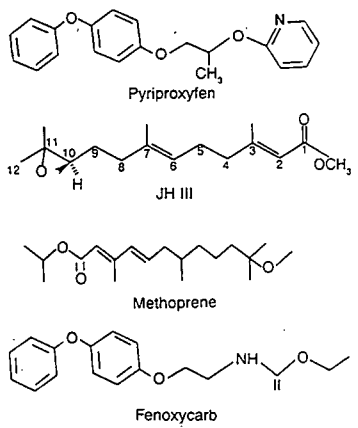

Figure 1. Structural formulas comparing differences between juvenile hormone and juvenile hormone mimics.

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MATERIALS AND METHODS

Fleas used in these studies were obtained from a laboratory colony maintained at Texas A&M University. For adult mortality studies, unfed fleas were collected during the first 2 days after emergence and were continuously exposed to treated filter paper as 6 replicate groups of 20 fleas each. Fleas maintained on dog hair were fed warm (37°C) bovine blood through a parafilm membrane using a modified artificial dog feeding system similar to the one developed by Wade and Georgi (1988).

The membrane system was also used to obtain eggs of known age. Fleas removed from the blood source were transferred to clean cages. Females treated in this manner laid most of their eggs within 1 to 2 hours. By using several cages of fed fleas, relatively large numbers of eggs were collected simultaneously and exposed to treated filter paper to investigate the ovicidal and larvicidal effects of pyriproxyfen.

Histology sections used in necropsy studies were prepared with paraffin embedded fleas according to procedures of Palma *et al.* (1993). Sections were cut at a thickness of 7 microns and stained with Mallory-Heidenhain procedure of Cason (1950).

RESULTS

Effect of Pyriproxyfen Exposure on Unfed Adult Fleas

We investigated the modes of action of pyriproxyfen on adult fleas because newly emerged cat fleas are resistant to starvation and can live several weeks without feeding. This suggested that cat fleas might undergo a reproductive diapause similar to that found in many other adult insects. Since adult diapause is usually caused by the absence of JH, we reasoned that pyriproxyfen might mimic the effect of JH and cause the premature termination of diapause. In turn, this might cause rapid starvation of the unfed flea.

We tested this hypothesis by exposing adult fleas to filter paper treated with pyriproxyfen. The dry papers were placed in gauze-covered jars and held in a humidified room. Fleas were confined to the papers continuously for 10 days to determine whether pyriproxyfen exposure caused mortality.

Results of this experiment (Fig. 2) showed that fleas on the pyriproxyfen treated papers began to die at an accelerated rate after 4 days. Mortality continued at a much higher rate in the treated

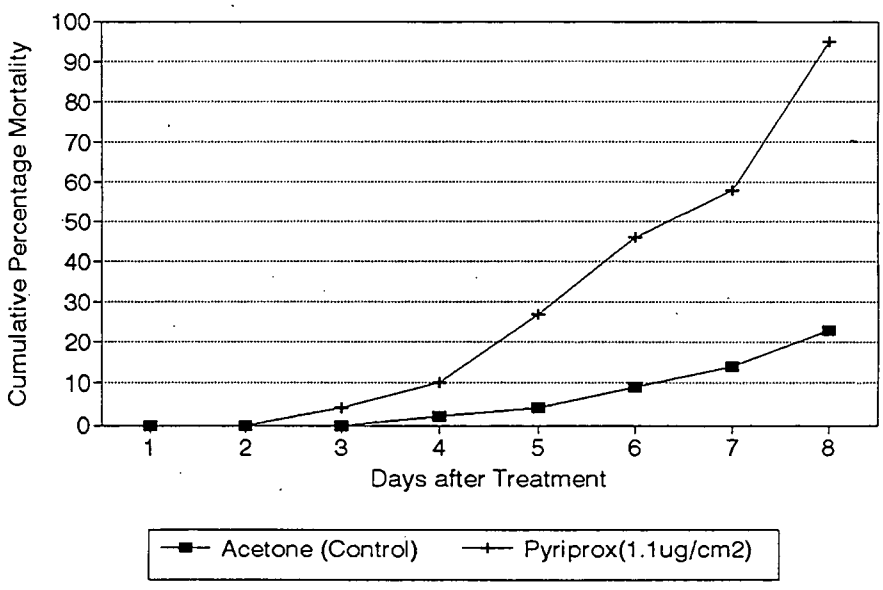

Figure 2. Mortality of unfed adult fleas following exposure to acetone or pyriproxyfen-treated filter papers.

Figure 3. Sagittal section through the abdomen of an unfed flea after 5 days of exposure to pyriproxyfen-treated filter paper. Centre area (G) is the air distended midgut which is occupying space normally filled with fat body, 202x

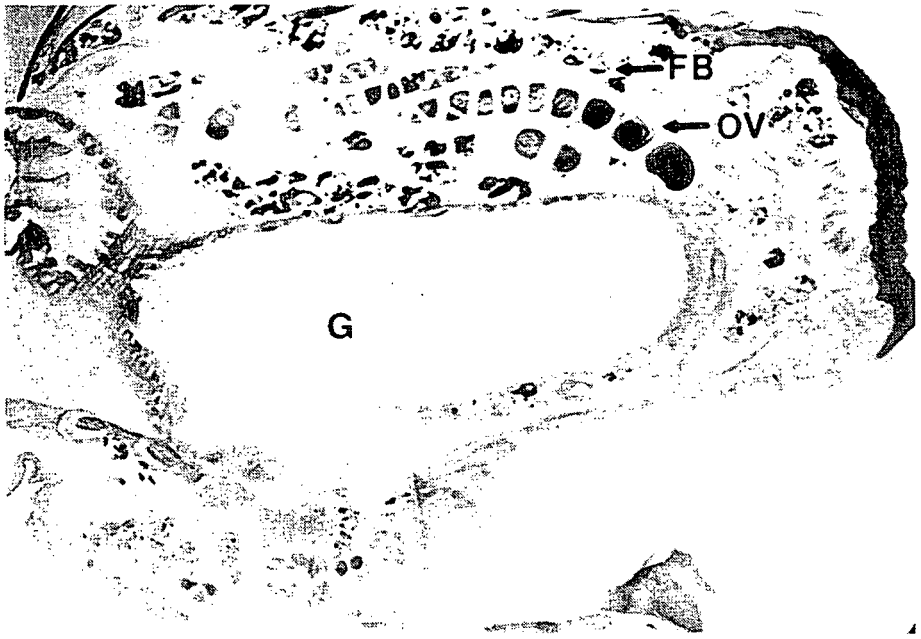

Figure 4. Sagittal section through the abdomen of an unfed control flea after 5 days exposure to acetone-treated filter paper. Lower centre (G) is the undistended midgut. Dorsal area above midgut contains extensive deposits of fat body (FB) and ovarioles (OV), 225x.

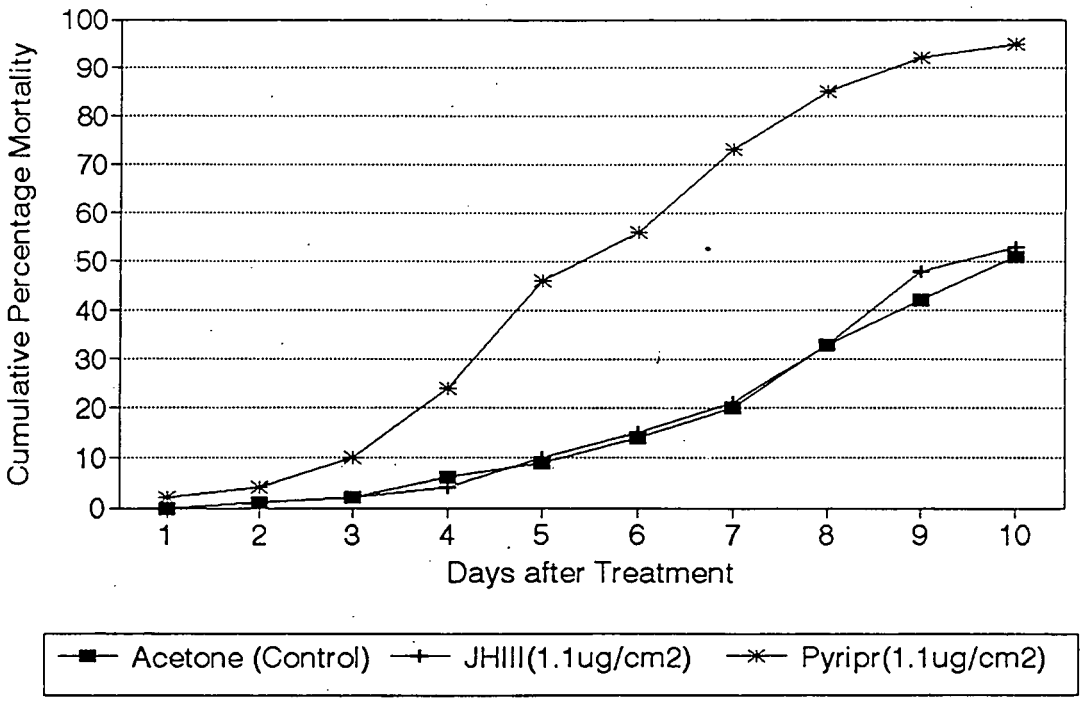

Figure 5. Mortality of unfed adult fleas exposed following exposure to filter papers treated with acetone, Juvenile Hormone III, or pyriproxyfen.

group compared with the controls. By day 8, 96% of the fleas exposed to pyriproxyfen had died. Control mortality was also fairly high at 20%, but significantly less than the treated group based on the statistical test for standard error of the percent difference (Franzblau, 1958).

Several fleas from the pyriproxyfen and control groups were examined histologically to determine whether either group showed evidence of starvation. Slides made from pyriproxyfen treated fleas showed that most of the fat body cells had disappeared and that the midgut was distended with air (Fig. 3). In comparison, control fleas still contained fat body reserves and showed no evidence of midgut distention (Fig. 4).

These results alone did not prove that pyriproxyfen caused death by mimicking the action of JH. Indeed our subsequent experiments contradicted this hypothesis.

To determine whether death was due to juvenile hormone, we repeated this experiment using filter papers treated with pyriproxyfen and JH III. Control papers treated with acetone alone were also included for comparative purposes. The results (Fig. 5) again showed that pyriproxyfen killed most of the adult fleas over a 10 day exposure period. However, JH exposure was no more toxic than the acetone control, indicating that flea mortality associated with pyriproxyfen exposure was not due to a JH effect.

Affect of Pyriproxyfen Exposure on Fed Adult Fleas

We then examined the hypothesis that death of pyriproxyfen treated fleas was due to starvation. By using the artificial membrane system (Fig. 6), we were able to expose adult fleas to pyriproxyfen residues on dog hair while they fed on blood through the parafilm membrane (Fig. 7). If pyriproxyfen caused death by starvation, feeding fleas would presumably be unaffected by pyriproxyfen exposure.

Results of this experiment (Fig. 8) showed that pyriproxyfen killed blood fed fleas at about the same rate as it had killed unfed fleas in our earlier tests. Control fleas on the other hand were unaffected when exposed to acetone treated dog hair. Since both the pyriproxyfen and the control fleas produced several hundred eggs during the course of this experiment, the treated fleas obviously died even though they fed on blood.

Based on these results, it is obviously premature to conclude that pyriproxyfen-treated fleas die from starvation. However, it is interesting to note that most of the eggs laid by pyriproxyfen-treated

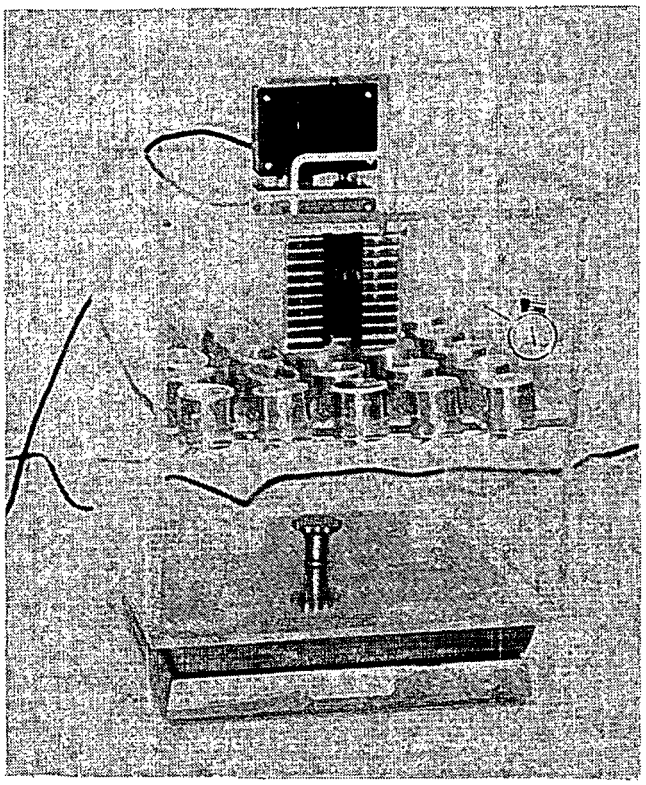

Figure 6. Synthetic membrane feeding system for adult fleas showing heated chamber and blood-filled feeding cylinders. Apparatus is sold under the name "Artificial Dog" by Flea Data, Inc., Freeville, NY, 6x.

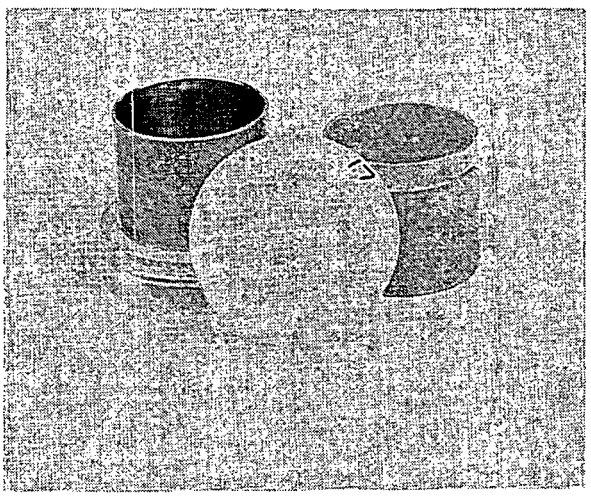

Figure 7. Close up of feeding cylinders; membrane covered by cylinder on right, inverted cylinder is flea cage on left. Center cage showing dog hair substrate, 2.4x.

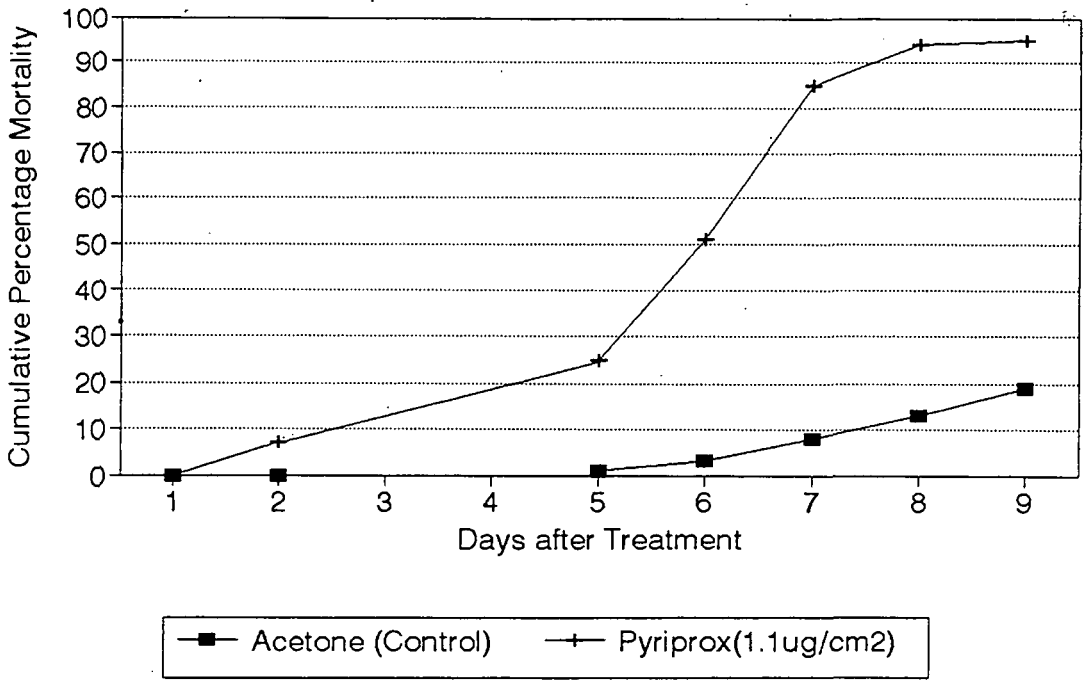

Figure 8. Mortality of blood-fed adult fleas following exposure to acetone or pyriproxyfen-treated filter papers.

fleas do not contain yolk. Therefore, it may be that pyriproxyfen interferes with the fleas ability to synthesize protein. In which case death may indeed be due to starvation.
Further research on the mode of action of pyriproxyfen is continuing because JH mimics are generally non-toxic to adult insects except at higher concentrations.

Sensitivity of Flea Eggs to Pyriproxyfen Residues

Marchiondo *et al.* (1990) reported that cat flea eggs exposed to fenoxycarb residues failed to complete embryogenesis, and that even 1 minute exposure periods caused embryocidal effects. In similar studies, we exposed eggs to pyriproxyfen treated filter paper to determine whether it also influenced hatching of cat flea eggs. Acetone treated filter paper served as the control. The dosage of pyriproxyfen tested 1.1 µg/cm was the same as that used by Marchiondo *et al.* with fenoxycarb. Results of this study (Table 1) showed that cat flea eggs were less sensitive to pyriproxyfen than they were to fenoxycarb. Sixty second exposure of eggs to pyriproxyfen had a relatively minor effect on percent hatch regardless of the age of the egg after oviposition. Some reduction in percent hatch occurred when 0-4 hour-old eggs were exposed to pyriproxyfen, but 24 hour and 48 hour-old eggs exposed for 60 seconds were not affected. In contrast, Marchiondo *et al.* found that all eggs exposed for 60 seconds to fenoxycarb died even those that were 48 hours-old before exposure.

Exposure of eggs of different age groups showed that the sensitivity of flea eggs to pyriproxyfen increased with time of exposure (Table 1). For example, none of the larvae hatched from the 0-4 hour egg group when they were exposed to pyriproxyfen for 2 hours. Two hour exposure was also much more effective in preventing hatching of 24 and 48 hour old eggs than shorter exposure intervals.

Results of this experiment showed that sensitivity of flea eggs to pyriproxyfen also decreased with age after oviposition. Only 25% of the eggs from the 0-4 hour group hatched after 1 hour of exposure compared with 66 and 90% hatch respectively in the 24 to 48 hour age groups.

When we reared larvae hatching from pyriproxyfen-treated eggs, we found that many of them died in the first instar (Table 2). Even those eggs exposed for 60 seconds were affected and 10 minute exposure resulted in 50% mortality in first instar larvae. Likewise, older eggs exposed for 35 to 50 minute intervals showed this latent toxic effect in newly hatched larvae. In contrast, those larvae that survived the first instar were apparently unaffected by pyriproxyfen treatment of the egg. Most of these larvae went on to develop to adult fleas.

Table 1. Percentage hatch of flea eggs exposed to pyriproxyfen (1.1 µg/cm) or acetone (control) treated filter paper.

Age of Eggs at Treatment	Length Exposure Pyriproxyfen	No. of Eggs	No. Larvae Hatching	Percentage Hatch
0-4 hr	None	89	79	89
	60 sec	26	19	73
	10 min	125	50	40
	15 min	31	10	32
	30 min	42	10	24
	60 min	48	12	25
	2 hr	82	0	0
	4 hr	167	1	1
24 hr	None	25	19	76
	60 sec	25	17	68
	15 min	52	36	69
	30 min	54	45	83
	35 min	214	138	64
	60 min	59	39	66
	2 hr	70	6	9
48 hr	None	24	19	79
	60 sec	24	19	79
	15 min	54	50	93
	30 min	42	36	86
	50 min	164	140	85
	60 min	51	46	90
	2 hr	85	22	26

Table 2. Mortality of larvae hatching from flea eggs exposed to pyriproxyfen or acetone (control) treated filter paper

Age of Eggs at Treatment	Length of Exposure to Pyriproxyfen	No. Eggs	No. Larvae Hatching	Percent Hatch	% Mortality in 1st Instar	No. 2nd and 3rd Instars
Control	None	189	144	76	6	135
0-4 hr	60 sec	230	161	70	23	124
	10 min	137	48	35	50	24
24 hr	35 min	261	157	60	44	88
48 hr	50 min	142	125	88	49	64

DISCUSSION

The new modes of action of pyriproxyfen described in this report are poorly understood. For example, we do not know how pyriproxyfen causes mortality in adult fleas or why exposure of young eggs to pyriproxyfen eventually kills the developing larvae.

Adult mortality which we originally thought was due to starvation may be caused by some other affect, because fed fleas die when exposed to pyriproxyfen. Death of the adult is not immediate but requires several days of exposure to pyriproxyfen during which fleas are able to produce some eggs. Therefore, even though fleas can digest blood, the fact that no yolk is deposited in the eggs suggests that they may not be able to synthesize protein needed for survival.

The relationship between age of flea eggs and their sensitivity to pyriproxyfen was similar to that reported earlier in studies on the ovicidal effects of methoprene (Olsen, 1985). However, unlike methoprene, pyriproxyfen exposure of flea eggs for intervals of less than 2 hours often caused mortality of first instar larvae.

Marchiondo et al. (1990) found that cat fleas hatched from fenoxycarb-treated eggs, only if the eggs were treated after the completion of embryogenesis. In contrast we found that fleas often completed larval development in pyriproxyfen-treated eggs even though they were exposed to pyriproxyfen as early as 4 hours after oviposition.

As reported by Marchiondo *et al.*, many of the larvae hatching from treated eggs died in the first instar. According to Marchiondo *et al.* death from fenoxycarb was caused by rupture of the larval

gut. In contrast, we found no evidence of gut rupture in larvae hatching from pyriproxyfen treated eggs, indicating that another mode of action was responsible for death of first instar larva. The unusual feature of this mode of action was that death of the first instar larvae could be programmed by exposure of eggs to pyriproxyfen up to 3 days earlier.

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