

Effects of the Insect Growth Regulator, Methoprene, on *Onthophagus taurus* (Coleoptera: Scarabaeidae)

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ABSTRACT A bioassay was conducted to determine the impact of methoprene, an insect growth regulator (IGR), on fecundity, larval survival, and size of progeny for *Onthophagus taurus* Schreber. Adult *O. taurus* dung beetles were offered methoprene-treated manure in three to five replications each at concentrations of 0.08, 0.45, and 4.5 ppm, respectively. An additional group of adult beetles was immersed in a methoprene–water solution and allowed to reproduce in containers with untreated manure. Data from all treatment groups were compared with untreated control groups. Methoprene did not seem to hinder brood production at 0.45 ppm. Survival of *O. taurus* was not affected by methoprene-treated manure at 0.08 ppm or when parent beetles were immersed in methoprene–water solution. However, progeny survival was significantly reduced on manure treated with methoprene at 4.5 ppm. Mean pronotal width of *O. taurus* progeny was significantly smaller in beetles fed methoprene-treated manure (4.5 ppm). The low dose of 0.08 ppm did not affect pronotal widths nor did topical application of methoprene to adults affect pronotal widths in resulting offspring. Although some adverse effects of methoprene were observed at higher concentrations, use of methoprene at concentrations of 0.08 ppm as part of a horn fly control program likely would not greatly affect populations of *O. taurus*, the most common paracoprid dung beetle in North Carolina.

KEY WORDS *Onthophagus taurus*, dung beetles, methoprene, insect growth regulator

The horn fly, *Hematobia irritans* L., is a blood-feeding dipteran of significant importance to the cattle industry (Drummond et al. 1981, Watson et al. 2002). Traditionally, control of horn fly included the use of insecticides applied as dusts, sprays, pour-ons, boluses, feed additives, and insecticide-impregnated ear tags (Butler and Okine 1999). Insecticide resistance has made horn fly control difficult (Miller et al. 1983, Quisenberry et al. 1984, Sheppard 1984, Hogsette et al. 1991, Sheppard and Joyce 1992). In the last 30 yr, macrocyclic lactone insecticides such as ivermectin, abamectin, and eprinomectin have been used effectively against pyrethroid-resistant horn flies. However, a negative impact on nontarget arthropods, particularly dung beetles, has been documented because of residues in manure (Floate et al. 2005, Wardhaugh 2005). To manage resistance in horn flies and reduce negative impact on beneficial insects, entomologists recommend rotating between insecticide classes and selecting insecticides that are more compatible with dung beetles. For example, the macrocyclic lactone insecticide, moxidectin, had only $\approx 1.6\%$ of the toxicity for *Onthophagus gazella* (F.) compared with abamectin (Doherty et al. 1994).

Another class of insecticides meriting consideration for this use pattern is the insect growth regulators (IGRs) including diflubenzuron, fluazuron, cyromazine, and dicyclanil (Floate et al. 2005). Methoprene (isopropyl (*E,E*)-(*RS*)-11-methoxy-3,7,11-trimethyl-2,4-dienoate), a juvenile hormone (JH) analog, is an IGR that disrupts normal insect development. JH is necessary for insects to molt and reach the next stage in their development. With each stage, the amount of JH is reduced and insect eventually develops into an adult. The constant presence of methoprene prevents the targeted insect from ever maturing. It is considered to be nontoxic to mammals and therefore it is available for use as a feed-through insecticide. At 7.5–10 ppm, methoprene readily controls house flies (Breedon et al. 1981). However, at concentrations of <0.08 ppm, methoprene has been successfully used for control of the immature stages of horn fly developing in manure (Blume et al. 1974, Fincher 1991). Methoprene can be administered orally either as a bolus (1.5 and 3.0%), mineral supplement (10.5%), feed additive (0.4, 1.0, 2.0, and 10.5%), or directly in the drinking water of cattle (Blume et al. 1974, Harris et al. 1974, Beadles et al. 1975, Fincher 1991). The percentage of active ingredient present in formulated products varies greatly (U.S. EPA 2001). Once consumed by cattle, $\approx 40\%$ of the total administered dose is recovered in feces as unmetabolized methoprene (Chamberlain et al. 1975). For relative ease of application, methoprene

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formulated as mineral supplements or feed additives are favored over boluses among producers to achieve the 0.08 ppm concentration in fresh cattle feces for horn fly control.

Published accounts suggest that certain dung beetle species are less susceptible to methoprene than others. Manure from steers treated with methoprene boluses (3%) was presented to two dung beetle species: *O. gazella* and *Sisyphus rubrus* Pashalidis (Fincher 1991). There was no significant difference in number of brood balls constructed or the number of emerging adults between beetles provided manure containing methoprene and those offered untreated manure. In contrast, emergence of horn flies from the treated manure was reduced by 95.3% (Fincher 1991). In a field study, the number of Scarabaeidae from the genus *Aphodius* was significantly reduced when fresh cattle dung pats were collected 12 d after administering 3% (30,000 ppm) methoprene boluses compared with dung pats from untreated cattle (Watson et al. 1986).

Methoprene effects on *O. gazella* survival are dose and life stage dependent. When methoprene was mixed directly into manure at a concentration of 100, 10, 5, or 1 ppm, it inhibited egg hatch in *O. gazella* by 100, 56, 33.3, and 8.7%, respectively (Blume et al. 1974). Manure from a steer treated with methoprene at a rate of 1 mg/kg body weight also inhibited egg hatch up to 32.6%, but there was no apparent effect on surviving larvae or on adults (Blume et al. 1974).

Onthophagus taurus Schreber is a very common and successful paracoprid dung beetle originating from the Middle East, Europe, and North Africa. This beetle first appeared in Florida, where it was accidentally introduced in 1971; it was subsequently collected in Georgia and southeast Alabama (Fincher and Woodruff 1975). Later collections showed *O. taurus* presence in Mississippi, Louisiana, and South Carolina (Fincher et al. 1983), as well as California and Missouri (Macrae and Penn 2001). *O. taurus* is the most common dung beetle in Piedmont and coastal regions of North Carolina, comprising 60% of the total dung beetle fauna at those two sites (Bertone et al. 2005). Cattle producers, recognizing the importance of this insect for manure reduction in the North Carolina pasture ecosystem, seek horn fly control strategies that are compatible with dung beetle populations.

Topical application of methoprene (400 mg/kg larval weight) to larvae of *O. taurus* delayed the onset of metamorphosis during the first critical period (6–10 d before pupation) (Emlen and Nijhout 1999, 2001). As a result of this treatment, the number of male *O. taurus* developing horns was reduced, and males were smaller relative to untreated larvae. When methoprene was administered during the second sensitive period toward the end of the third instar, males destined to become minor males (small horns) produced large horns in 80% of the cases (Emlen and Nijhout 1999, 2001). From those studies, it is obvious that methoprene at sufficient doses alters development of dung beetles. However, it is not clear if methoprene at the concentrations of 0.08 ppm found in the manure

necessary for horn fly control adversely impacts *O. taurus*, the most common pasture inhabiting dung beetle in North Carolina. The objective of this study was to evaluate the effects of methoprene on fecundity of *O. taurus* either when fed manure containing methoprene at different concentrations or when methoprene was topically applied to adult parent beetles. We evaluated survival rate and size of *O. taurus* progeny from brood balls made from methoprene-treated manure and from topically treated adults.

Materials and Methods

Adult *O. taurus* beetles were collected with dung baited pitfall traps (Bertone et al. 2005) from a wild population not treated with any insecticides at the Center for Environmental Farming Systems (CEFS) in Goldsboro (Wayne Co.), NC. Beetles were separated by sex and held in 2-liter plastic containers filled with sifted and moistened Black Kow soil (Black Gold Compost, Oxford, FL). Containers were covered with mesh tops and held at a temperature of 27°C with a 16:8-h light:dark regimen.

Manure used in these experiments was collected from a pesticide-free dairy herd and frozen to kill any insects present in the dung. Before use, the manure was thawed overnight at room temperature and mixed to a homogenous consistency. Each treatment replicate consisted of 300 g cow manure and five male-female pairs of *O. taurus* beetles.

Technical grade methoprene (Wellmark International, Schaumburg, IL) was added to manure to achieve concentrations of 0.08, 0.45, and 4.5 ppm for dung wet weight. Topical application was achieved by mixing 5.96 μ l of 23% methoprene in 2 ml of water (680 ppm). Groups of 10 adult beetles (five pairs) were separately immersed in the methoprene–water solution for 20 min and placed in pots with untreated manure. Data from all methoprene treatments were compared with their respective untreated control groups.

Experimental units were 3.8-liter pots (filled with sifted Black Kow soil up to 4 cm of the rim). There were a total of three trials for 0.08 ppm, four for 0.45 ppm, and five for 4.5 ppm treatment groups. Five pots per treatment were used in each of the trials, and each pot contained five male-female beetle pairs with 300-g aliquot of treated or untreated manure. To prevent beetle escape, pots were covered with hairnets held in place with rubber bands. Pots were held at 27°C with a 16:8-h photoperiod. Adult beetles were given 2 wk to produce brood balls, after which they were removed from the pots together with constructed brood balls. Brood balls were counted and placed by treatment in 2-liter plastic containers with 1 cm of Black Kow soil on the bottom. A maximum of 10 brood balls were placed on the soil and covered with additional 2 cm of soil. This process was repeated until all the brood balls were allocated. After all the brood balls were placed in the container, they were sheltered with 4 cm of soil and covered with a paper towel and a screened top. Containers were moistened with tap water periodi-

Table 1. Mean \pm SEM no. of brood balls produced by adult *O. taurus* and progeny percent survival in the methoprene-treated manure (0.08 ppm) and the untreated control

Trial	No. brood balls		Percent survival	
	Control	Methoprene	Control	Methoprene
1	15.6 \pm 3.4	11.4 \pm 2.0	73.9 \pm 10.0	57.4 \pm 10.4
2	11.3 \pm 2.9	4.2 \pm 1.3	25.4 \pm 5.7	35.1 \pm 6.5
3	4.0 \pm 1.2	2.7 \pm 0.9	55.6 \pm 5.5	47.2 \pm 23.8
Mean	11.3 \pm 1.8a	6.6 \pm 1.7b	53.1 \pm 7.5a	46.5 \pm 7.2a

Means followed by the same letter within a row for brood ball production and percent survival are not significantly different at $\alpha = 0.05$ (brood balls: $F = 5.15$; $df = 1,19$; $P = 0.04$; survival: $F = 0.39$; $df = 1,21$; $P = 0.54$).

cally and equally to prevent desiccation of the soil and brood balls. After 4 wk, emerged beetles were counted, and all brood balls were checked for the presence of teneral adults. The numbers of brood balls and the percentages of adult emergence were compared between each treatment and its respective control group. Progeny were compared by size between treatment and control groups measuring the pronotal width using electronic calipers (Fowler 54-100-330, Euro-Cal 6/150 mm, Fred V. Fowler Co. Inc. Newton, MA) (Hunt and Simmons 2000).

Statistical analyses were performed using SAS 9.1.3 (SAS Institute 2001). Raw data were square root transformed for analysis using analysis of variance (ANOVA; PROC GLM statement) to compare the number of brood balls among different treatments ($\alpha = 0.05$). Means were compared using Tukey's studentized range test. A mixed model ANOVA (PROC MIXED statement) was used to compare the mean percentage emergence among different treatments taking into account the number of brood balls produced in different treatments ($\alpha = 0.05$). To determine whether the results changed, the analysis was repeated when the pots producing zero brood balls were removed from the data set. ANOVA (PROC GLM statement) was used to compare mean pronotal widths for beetles emerging from different treatments ($\alpha = 0.05$).

Results and Discussion

Onthophagus taurus exposed to methoprene-treated manure (0.08 ppm) produced between 0 and 18 brood balls. Beetles in the untreated manure produced between 0 and 25 brood balls per container. Mean number of brood balls produced by beetles exposed to the low dose of methoprene (6.6 \pm 1.7) was significantly less than untreated control (11.3 \pm 1.8; Table 1). The difference is of concern, but it is not alarming because the number of brood balls has varied greatly in previous studies conducted under laboratory conditions (Blume and Aga 1975, Fincher 1991, Bertone et al. 2006). Furthermore, the percent survival was not significantly different for the methoprene-treated group (46.5 \pm 7.2) compared with control (53.1 \pm 7.5; Table 1).

Table 2. Mean \pm SEM no. of brood balls produced by adult *O. taurus* in the methoprene-treated manure (0.45 ppm) and the untreated control

Trial	Control	Methoprene
1	5.2 \pm 0.6	5.0 \pm 1.6
2	12.6 \pm 2.6	10.6 \pm 1.9
3	7.2 \pm 1.6	7.2 \pm 3.0
4	0.6 \pm 0.6	2.6 \pm 1.2
Mean	6.4 \pm 1.8a	6.4 \pm 1.8a

Means followed by the same letter within rows for the no. of brood balls are not significantly different at $\alpha = 0.05$ ($F = 0.00$; $df = 1,32$; $P = 0.97$).

Because of a mite infestation, the brood within the manure treated with 0.45 ppm of methoprene was unable to complete development so realistic measures of survivability were not possible.

Numbers of brood balls produced between methoprene at 0.45 ppm and control groups were not significantly different at 0.45 ppm (Table 2). Control beetles made between 0 and 21 brood balls (6.4 \pm 1.8), whereas number of brood balls produced by methoprene-exposed beetles ranged between 0 and 18 per container (3.6 \pm 2.6). Similarly Fincher (1991) did not find a significant difference in *O. gazella* brood production when fed methoprene-treated or untreated manure. A mite infestation prevented brood in the 0.45-ppm trial from completing development.

There was a significant difference in brood ball production between the untreated control and methoprene-treated manure (4.5 ppm; Table 3). There was no significant difference in the number of brood balls between control and methoprene topically applied to the adults, as well as methoprene-treated manure and topical application (Table 3). However, the number of brood balls in both methoprene treatments was lower than in the untreated control. Number of brood produced by control beetles ranged from 5 to 51 (31.3 \pm 2.3). Adults in methoprene-treated manure (4.5 ppm) produced 0–55 brood balls (21.4 \pm 2.8), and beetles with topical application of methoprene produced 0–56 brood balls (25.8 \pm 4.4).

Some pots containing dung beetles produced no brood balls regardless of treatment (control and 0.08, 0.45, and 4.5 ppm). When the pots not producing any brood balls were removed from the analysis, significance levels were unchanged.

Percent survival of larvae feeding on methoprene (4.5 ppm)-treated manure (39.4 \pm 6.2) was significantly lower compared with larvae exposed to the untreated manure (74.2 \pm 7.9), but there was no difference compared with larvae from the topical methoprene treatment (55.8 \pm 8.6; Table 3).

Pronotal widths of 115 adult progeny were measured for the 0.08 ppm treatment. Mean pronotal width was not significantly different in *O. taurus* progeny fed on methoprene-treated manure, suggesting that methoprene at this concentration did not have a negative impact on the size of surviving *O. taurus* (Table 4). However, mean pronotal width was significantly lower for the progeny fed 4.5 ppm methoprene-treated manure compared with the untreated

Table 3. Mean ± SEM no. of brood balls produced by adult *O. taurus* and the progeny percent survival in the methoprene-treated manure (4.5 ppm), the methoprene topical application, and the untreated control groups

Trial	Brood balls			Percent survival		
	Control	Methoprene	Topical	Control	Methoprene	Topical
1	44.2 ± 3.0	28.8 ± 6.9	18.2 ± 9.2	76.8 ± 2.9	45.4 ± 9.7	69.3 ± 10.8
2	39.6 ± 4.6	17.4 ± 3.5	32.2 ± 4.1	71.5 ± 11.2	27.0 ± 6.8	62.0 ± 8.0
3	9.2 ± 1.3	7.4 ± 1.0	15.0 ± 4.6	94.1 ± 2.5	64.2 ± 7.1	32.4 ± 9.1
4	32.4 ± 7.6	28.6 ± 5.3	36.6 ± 7.1	77.0 ± 9.5	5.3 ± 0.9	54.4 ± 7.0
5	31.2 ± 3.6	25.0 ± 8.0	27.2 ± 7.5	51.7 ± 9.3	14.5 ± 0.0	60.8 ± 4.8
Mean	31.3 ± 2.3a	21.4 ± 2.8b	25.8 ± 4.4ab	74.2 ± 7.9a	39.35 ± 6.2b	55.8 ± 8.6ab

Means followed by the same letter within a row for the no. of brood balls and percent survival are not significantly different at $\alpha = 0.05$ (brood balls: $F = 3.34$; $df = 2,60$; $P = 0.04$; survival: $F = 6.15$; $df = 2,112$; $P = 0.02$).

control or progeny of the topically exposed parents (Table 4).

In this experiment, application of methoprene at the label concentration recommended to kill horn flies (0.08 ppm in fresh manure) did not have a negative effect on brood survival of *O. taurus*. Fecundity seemed to be affected, but under laboratory conditions in this and other studies, the brood ball numbers varied greatly (Blume and Aga 1975, Fincher 1991, Bertone et al. 2006). Adults produced between 0 and 56 brood balls with an average of 12.14 ± 2.8 brood balls per pot or 2.48 per adult pair. These results were similar to a previous study in which eight pairs of *O. taurus* given 550 g of manure produced an average of 11.7 ± 1.8 brood balls in coastal plains sandy loam and 13.7 ± 5.3 in washed sand (Bertone et al. 2006). However, our observed brood numbers were lower than the averages produced by isolated *O. taurus* pairs over a 14-d period (Hunt and Simmons 2002). Those authors held beetle pairs separately, preventing competition for food resources, which may explain the higher number of brood. Furthermore, in this study, although there was no significant difference between the number of brood balls produced by the control beetles and those exposed to methoprene at 0.45 ppm, this difference was significant for the control and beetles exposed to methoprene-treated manure at 4.5 ppm. These inconsistencies lead us to believe that differences in brood ball production were caused by factors other than methoprene, for example, competition for food resources. This also begs the question of brood production as a reliable indicator of dung beetle fe-

cundity, specifically in laboratory studies. Reproductive variability observed in this and other studies reflects a k-selection strategy for the natural history of dung beetles. Such variability makes it difficult to fully understand the impact of toxins in the dung environment without established toxicity testing standards (DOTTS 2005).

Susceptibility of dung beetles to methoprene may vary with species and developmental stage. In a previous field study, a 3% (30,000 ppm) methoprene bolus application significantly reduced the number of Scarabaeidae, mostly belonging to the genus *Aphodius* (Watson et al. 1986). However, Pickens and Miller (1975) found no significant negative impact of methoprene on *Aphodius fimetarius* L. inhabiting dung pats collected from methoprene-fed cattle at the dose of 2.5 mg/kg cattle weight. This apparent variability among genera and species warrants further study.

When applied to ventral abdominal segments of male pupae of *Oryctes rhinoceros* L., methoprene (20, 50, and 100 μg or ≈ 2.5 , 6.25, and 12.5 ppm) inhibited normal development of the male reproductive system (Jacob 1989), whereas in this study, topical methoprene application to sexually mature *O. taurus* adults did not seem to inhibit their reproductive ability. In addition, rhinoceros beetle pupae died after applying methoprene topically or through injection at 125, 25, and 5 ppm (Dhondt et al. 1976). However, application of methoprene in concentrations as high as 50 ppm to adult sawtoothed and merchant grain beetles did not cause mortality or reduced fecundity (Strong and Diekmann 1973). No mortality was recorded when adult beetles belonging to families Cucujidae and Tenebrionidae were exposed to methoprene at concentration of 20 ppm (Pierce et al. 1986). Our data suggest that *O. taurus* responds similarly to methoprene as do the grain beetles, cucujids, and tenebrionids.

We found that methoprene-treated manure at a concentration (0.08 ppm) sufficient for horn fly control did not inhibit development of *O. taurus*. Similarly, manure from cattle treated with 3% (30,000 ppm) methoprene boluses had no significant negative effect on brood ball construction and adult emergence of *O. gazella* and *Sisypus rubrus* Paschalidis compared with an untreated control (Fincher 1991). However, when methoprene (52.5% EC) was applied to manure at concentrations of 100, 10, 5, and 1 ppm, *O. gazella*

Table 4. Mean ± SEM pronotal widths of the adult progeny developed on methoprene-treated manure (0.08 and 4.5 ppm), progeny from adults treated topically with 4.5 ppm methoprene, and respective untreated controls

Treatment	4.5 ppm		0.08 ppm	
	Female	Male	Female	Male
Control	4.64 ± 0.04a	4.67 ± 0.04a	4.64 ± 0.04a	4.60 ± 0.08a
Manure	4.32 ± 0.08b	4.33 ± 0.08b	4.64 ± 0.07a	4.85 ± 0.13a
Topical	4.67 ± 0.06a	4.67 ± 0.06a	NA	NA

Means followed by the same letter within columns are not significantly different at $\alpha = 0.05$: 0.08 ppm (for treatment: $F = 0.11$; $df = 1,86$; $P = 0.75$; for sex: $F = 1.70$; $df = 1,86$; $P = 0.20$); 4.5 ppm (for treatment: $F = 13.65$; $df = 2,636$; $P < 0.0001$; for sex: $F = 0.21$; $df = 1,636$; $P = 0.65$).

NA, not applicable.

development was inhibited by 100, 56, 33.3, and 8.7%, respectively (Blume et al. 1974). All these concentrations were above the concentration (0.08 ppm) sufficient to kill the target insect, horn fly. Similarly, the concentration of 4.5 ppm methoprene used in this study negatively affected *O. taurus* survival.

Two factors most influence *O. taurus* size—food quality and food availability—with little difference between sexes (Moczek 1998). In this study, there was no significant difference (0.08 ppm: $F = 1.70$; $df = 1,86$; $P = 0.20$; 4.5 ppm: $F = 0.21$; $df = 1,636$; $P = 0.65$) between male and female pronotal widths. However, this study showed that methoprene in the manure at a higher concentration (4.5 ppm) reduced feeding by *O. taurus* progeny. This was not surprising because topical application of methoprene resulted in inhibited normal male reproductive development (Jacob 1989) or pupal death in *O. rhinoceros* (Dhondt et al. 1976).

The ecological importance of IGR and parasiticide residues in animal feces on nontarget species is of great concern (Floate et al. 2005). Methoprene at a concentration (0.08 ppm) sufficient to kill horn flies did not have a negative effect on fecundity and progeny percent survival of *O. taurus*. However, this study does not address effects of methoprene on the many other insect species (Mohr 1943) that breed and live in cattle manure. This study suggests that methoprene used as a part of a horn fly control program would likely not substantially reduce population of the most common North Carolina paracoprid dung beetle, *O. taurus*.

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