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ABSTRACT A granular formulation of novaluron (Novaluron 0.2G, 0.2% [AI]), a newer benzoylphenyl urea insecticide, was evaluated for its efficacy in controlling the larval stage of horn flies, *Haematobia irritans* (L.); house flies, *Musca domestica* L.; and stable flies, *Stomoxys calcitrans* (L.), in cow manure. Various rates and insecticide placement locations (top, middle, and bottom of manure) were evaluated in this study and all combinations of these variables reduced adult emergence of all three species when compared with the untreated controls. The presence of deformed pupae indicated that novaluron had an insect growth regulator effect on the developing fly larvae. Top, middle, or bottom application rates of 0.125, 0.195, 0.25, and 0.375 g novaluron onto manure samples, reduced adult horn fly emergence by >90%. Middle and bottom application rates of 0.195, 0.25, and 0.375 g novaluron reduced adult house fly emergence >93%. All rates and placement combinations resulted in >98% reduction of adult stable fly emergence. The level of control efficacy observed against these three fly species along with the ease of use of a granular formulation, make this product an ideal candidate for use in an integrated livestock pest management program.

KEY WORDS *Haematobia irritans*, *Stomoxys calcitrans*, *Musca domestica*, benzoylphenyl urea

Horn flies, *Haematobia irritans* (L.) (Diptera: Muscidae), and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), are two of the most economically damaging ectoparasites of pastured cattle. Annual losses in cattle production because of reduced weight gain and poor feeding efficiency are estimated at over two billion dollars each year (Kunz et al. 1991, Taylor et al. 2012). House flies, *Musca domestica* L. (Diptera: Muscidae), are pestiferous to humans and cattle and can mechanically transmit, or serve as reservoirs for helminth eggs, bacteria, and viruses that cause enteric infections, eye infections, and skin infections (Greenberg 1973, Emerson et al. 1999, Sasaki et al. 2000).

Historically, filth fly management has been based on the use of insecticides applied as dusts, sprays, pour-ons, feed additives, ear tags, structure sprays, and boluses (Foil and Hogsette 1994, Butler and Okine 1999). Stable flies are particularly difficult to control because of the limited time they spend on hosts as well as the ability to disperse long distances (Taylor et al. 2010, Pitzer et al. 2011). Traditionally, stable flies were considered pests of confined cattle operations such as dairies and feedlots, but over the past few decades the use of round hay bales has complicated stable fly control in rangeland and pastured cattle operations. Round bale technology makes it more convenient for producers to feed pastured and rangeland cattle during winter months, but also increases amounts of wasted hay. This wasted hay mixes with cattle manure deposited at feeding sites and creates an ideal development medium for larval stable flies (Broce et al. 2005, Talley et al. 2009, Taylor and Berkebile 2011).

Reliance upon insecticides for fly control has led to an increased incidence of resistance that is the eventual cause of product failure. As the incidence of resistance increases and traditional chemical insecticides fail, the need is becoming crucial for compounds with novel modes of action that improve integrated pest management (IPM) programs.

Novaluron (1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxy-ethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea) is a newer insect growth regulator (IGR) in the benzoylphenyl urea group of insecticides and has very low toxicity to mammals, birds, and earthworms (Barazani 2001, Ishaaya et al. 2007). Novaluron is a

This paper reports the results of research only. Mention of a commercial or proprietary product in this paper does not constitute an endorsement by the U.S. Department of Agriculture. In conducting the research described in this report, the investigators adhered to protocol approved by the USDA–ARS Animal Welfare Committee. The protocol is on file at the USDA–ARS, Knipling-Bushland U.S. Livestock Insects Laboratory, Tick Research Unit, Kerrville, TX. 78028.

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chitin synthesis inhibitor that is active through ingestion and contact, in particular targeting larval insects that actively synthesize chitin. Previous work has shown that novaluron is larvicidal against sand flies and several species of mosquitoes (Mulla et al. 2003, Mascari et al. 2007, Arredondo-Jiménez and Valdez-Delgado 2006). A study conducted by Cetin et al. (2006) showed that a 1% emulsifiable concentrate of novaluron caused >80% mortality when fed to a field strain of house fly larvae, but no other work has been done to evaluate efficacy of novaluron against larval dipteran pests of cattle. The objective of the current study was to evaluate efficacy of a granular formulation of novaluron for controlling immature stages of horn flies, house flies, and stable flies in cow manure.

Materials and Methods

Fly Rearing. Flies used in this study were obtained from colonies of insecticide-susceptible horn flies, house flies, and stable flies reared at the U.S. Department of Agriculture–Agriculture Research Services (USDA–ARS), Knipling-Bushland U.S. Livestock Insects Research Laboratory (KBUSLIRL), Kerrville, TX. Laboratory colonies were maintained at 24–28°C, 60% RH, and a photoperiod of 12:12 (L:D) h. Colony horn fly larvae were reared on a cow manure and peanut hull diet (Lohmeyer and Kammlah 2006). Larval stable flies and larval house flies were reared on a diet of Purina Fly Chow (Purina Mills LLC, Gray Summit, MO). Colony adult horn flies and stable flies were fed citrated bovine blood, and adult house flies were fed a sugar/milk solution (50 g of powdered milk and 20 g of table sugar per liter of water).

Cattle Manure. Manure used for fly bioassays was collected from untreated steers maintained in stanchion at KBUSLIRL. Steers used for manure production weighed ≈136 kg and were fed 5.4 kg of 5.1 cm square alfalfa cubes per day and water ad libitum. Fresh manure was collected and placed in plastic bags and was frozen until needed for bioassays. Animal care procedures adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Institutional Animal Use and Care Committee of KBUSLIRL.

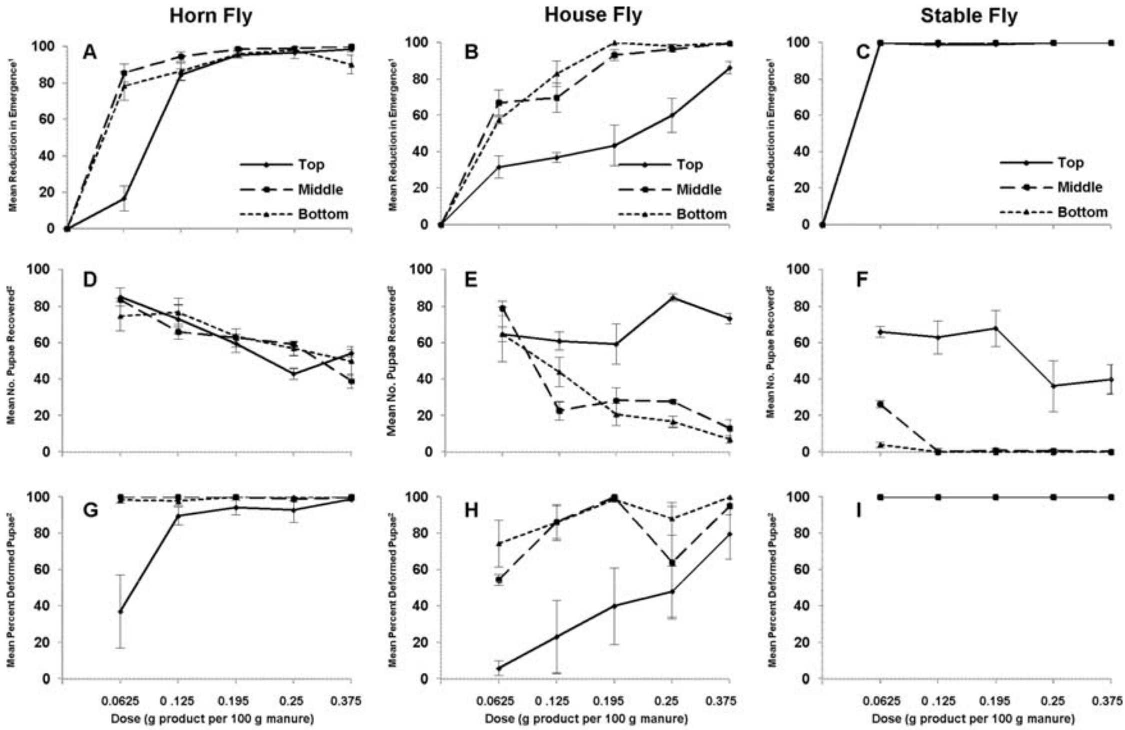
Larval Bioassays. Granular novaluron (MANA Novaluron 0.2G: a purple granule (0.4–1.68 mm) formulated as 0.2% [AI] in wood fiber/granite; Makhteshim Agan of North America, Inc, Raleigh, NC) was applied at five rates (0.0625, 0.125, 0.195, 0.25, or 0.375 g product per cup. In the field, a granular product for house and stable fly control would be scattered over areas where larval flies are suspected to be developing. In most cases the material may not remain on top, but may be worked down into the soil/manure to varying levels by animal movement or deposition of manure as cattle are standing around a food source. Therefore, three treatment placement locations were selected at each application rate to simulate field conditions: top of manure, product remains where it was originally placed; middle of manure, product ends up under ≈2.5 cm of manure; and bottom of manure, product ends up under ≈5 cm of manure. These treatment placement locations would also allow determination of how evenly novaluron would need to be distributed within manure for controlling larval horn flies developing in manure pats, should this product be formulated as a feed-thru product.

Each rate/location was applied to 100 g of manure contained in 532 ml plastic Solo brand cups (Solo Cup Company, Lake Forest, IL). For top treatment 100 g of manure were placed in the cup and the appropriate amount of granular novaluron was sprinkled on the top of the manure. For middle treatment 50 g of manure were placed in the cup, the novaluron was sprinkled on top, and the remaining 50 g of manure were added to the top. For bottom treatment: the appropriate amount of novaluron was sprinkled onto the bottom of the cup and 100 g of manure were added to the cup. A similar quantity of untreated manure was used as a control. Three replicates of each rate/location combination and untreated controls were performed. Bioassays for horn flies, house flies, and stable flies were initiated on separate days (14 September 2010, 26 October 2010, and 11 March 2011, respectively). After manure and product had been added to each cup, 100 fly eggs were applied to the top of the manure. Cups were covered with facial tissue held in place with a rubber band, maintained in a rearing room held at 28°C, 60% RH, and a photoperiod of 12:12 (L:D) h for 7–10 d, and observed for pupation. Pupae were then collected by flotation and numbers of normal and deformed pupae recorded. Pupae were held at 28°C, 60% RH, and a photoperiod of 12:12 (L:D) h until adult emergence. The number of emerged adults was recorded for each sample.

Statistical Analysis. Before analysis, manure bioassay data were corrected using Abbott's formula (Abbott 1925). Analysis of the corrected Abbott's formula variable was performed using the GLIMMIX procedure in SAS (SAS Institute 2009). The response variable (number of emerged adult flies) was nonnormally distributed in each bioassay, and was determined to follow the structure of the Beta probability distribution, which uses the logit link function as its linearization approach. To have complete estimability of the interaction and main effects, the Abbott's values were assessed in a 0–1 scale where values equal to 100% (i.e., complete control) were replaced with 9×10^{-9}. Rate, location, and their interaction were considered fixed effects in the statistical model. Estimated means, SEs, and differences of mean were calculated using the LSMEANS option. Multiple comparison P values were controlled for type I error using the Tukey–Kramer adjustment with $P < 0.05$.

Results

Horn Flies. All application rates and treatment locations of novaluron reduced adult fly emergence compared with the untreated controls (Fig. 1A). Statistical comparison of the treatments revealed a significant effect for the rate of novaluron applied ($F = 32.54$; df = 4, 29; $P < 0.0001$), the placement location of the novaluron



¹ Not raw data. Data were corrected with Abbott's formula (Abbott 1925) prior to analysis.
² Raw data.

Fig. 1. Effects of various rates and treatment placements of granular novaluron on adult emergence (A, B, C), number of pupae recovered (D, E, F), and pupal deformity (G, H, I) of horn flies, house flies, and stable flies.

($F = 16.18$; df = 2, 29; $P < 0.0001$), and the interaction of rate and placement location ($F = 5.86$; df = 8, 29; $P = 0.0002$) on adult fly emergence. Deformed pupae were observed at all application rates and treatment locations (Fig. 1G).

House Flies. All application rates and treatment locations of novaluron reduced adult fly emergence compared with the untreated controls (Fig. 1B). Statistical comparison of the treatments showed a significant effect for the rate of novaluron applied ($F = 46.28$; df = 4, 29; $P < 0.0001$), the placement location of the novaluron ($F = 111.04$; df = 2, 29; $P < 0.0001$), and the interaction of rate and placement location ($F = 10.48$; df = 8, 29; $P < 0.0001$) on adult fly emergence. As with horn flies, deformed pupae were observed at all application rates and treatment locations (Fig. 1H).

Stable Flies. All application rates and treatment locations caused ≥98% reduction in adult fly emergence (Fig. 1C). Deformed pupae were not observed for the bottom application rates of 0.0625, 0.125, 0.195, 0.25, and 0.375 g; however, it appears that mortality may have been caused in larval flies before they reached the point of pupation (Fig. 1I).

Discussion

Novaluron, formulated as a granule (0.2% [AI]) and applied to cow manure, is efficacious against larval horn flies, house flies, and stable flies in the laboratory. All of the rates and insecticide location placement combinations evaluated in this study reduced adult horn fly, house fly, and stable fly emergence. The presence of deformed pupae indicated that novaluron has an IGR effect on the development of the three fly species tested, resulting in larval mortality before they reached pupation or deformed pupae from which adults never emerged.

It was evident that novaluron middle and bottom application at >0.195 g affected both horn fly and house fly development, as this combination reduced adult emergence of these flies by >90%. Interestingly, a few of the more effective rate/placement location combinations for controlling adult horn fly emergence were not as effective against adult house fly emergence (i.e., middle application of 0.125 g novaluron and top application of 0.195, 0.25, and 0.375 g). This was not unexpected because these flies do not develop in similar environments in nature. Further, the efficacy we observed against house flies is similar to the efficacy observed by Cetin et al. (2006) for higher rates of a 1% emulsion concentrate formulation of novaluron mixed into larval house fly diet.

The granular formulation of novaluron evaluated in this study was particularly efficacious against stable flies. A dose affect was not apparent as all rates and placement combinations in this study exhibited >98%

reduction in adult stable fly emergence. This is an interesting phenomenon as the rate of insecticide needed to kill stable flies is usually much higher than the rate needed to kill horn flies (Frazar and Schmidt 1979, Schmidt and Kunz 1980, Miller et al. 1986). The IGR effect of novaluron on larval stable flies appears to be robust as few to no pupae were recovered from most rate and treatment placement combinations, and a very small number of stable flies survived to emergence.

In all likelihood, a granular product placed in the field would not remain on the surface of the treatment site, but would be worked down into the substrate by animal movement or deposition of manure, as is the case with wasted hay and other debris around cattle feeding sites. Though some variation in efficacy was observed for the different placement locations, it appears that the granular formulation of novaluron would work well regardless of where it is spread provided it is applied at a rate that is efficacious for controlling that particular fly species.

In conclusion, the granular formulation of novaluron used in this study appears to deliver adequate levels of active ingredient for effective control of horn flies, house flies, and stable flies in treated cow manure. The efficacy observed against stable flies is particularly promising, making this formulation of novaluron a viable candidate for use by cattle operations that use round bales. Future studies are being planned to evaluate granular novaluron in the field for use at round bale feeding sites and to evaluate longevity of efficacy. Studies are also needed to evaluate the potential of the product for controlling house flies developing in dairies and to determine treatment application techniques for using the product for horn fly control in the field.

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