Treatment of Pastures with Diflubenzuron Suppresses Horn Fly, *Haematobia irritans* (Diptera: Muscidae) Development[1]

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ABSTRACT Diflubenzuron is an insect growth regulator labeled for application to pastures and rangeland to suppress grasshopper (Orthoptera: Acrididae) populations. Livestock are permitted access to land immediately after treatment. We hypothesized the development and survivorship of horn fly *Haematobia irritans* (L.) larvae feeding on manure resulting from these animals would not be impacted due to the presence of diflubenzuron residue. Survivorship to the adult stage and percent pupae deformed were recorded for horn flies developing on manure samples from three pastures treated with 59 ml Dimilin 2L® /0.4 hectares. Pastures not treated served as the controls. Manure samples ranged in age from three to 31 d post treatment. This study was replicated in 2004 and 2005. Results were highly variable between site and year. Horn fly survivorship to the adult stage in 11 of 15 sample dates taken was lower in treated than the control manure samples. Accordingly, a significantly greater percentage of deformed pupae was recorded for samples from the treated than the control sites ≤17 d post treatment. Based on this study, our null hypothesis was rejected. Using Dimilin 2L to suppress pasture and range land pests can also impact horn fly populations associated with cattle feeding in these pastures. However, care should be taken to apply adequate pasture coverage ensuring appropriate Dimilin 2L® levels are consumed to achieve the suppression of associated horn flies.

KEY WORDS horn fly, *Haematobia irritans*, diflubenzeron

THE HORN FLY, *Haematobia irritans* (L.), (Diptera: Muscidae) is an important blood-feeding pest of cattle (Da Silva and Mendes 2002). Adults reside on the animal's surface, while their larvae develop in fresh cattle manure deposited in pastures (Lysyk and Steelman 2004). Insecticide applications to the animal's hide are the most common method implemented to suppress associated horn fly populations.

Insect growth regulators (IGR) are a class of insecticides that interfere with insect development (Da Silva et al. 2004). Diflubenzuron is an example of an IGR

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that interferes with chitin synthesis during molting (Garnett and Weseloh 1975), and it can suppress fly pest populations associated with cattle (Da Silva and Mendes 2002). Diflubenzuron applied under laboratory conditions at 1 mg/929 cm^2 to a breeding-surface area prevented the emergence of house flies, *Musca domestica* (L.) (Diptera: Muscidae), and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) (Wright 1974). Applications to the breeding area under field conditions at 50 mg/929 cm^2 resulted in 90% suppression of house flies (Wright 1974). House flies were suppressed by 97 and 91% when developing in manure produced by cattle given access to a mineral block containing either 0.1 or 0.05% respectively under laboratory conditions (Wright 1975). Horn flies were suppressed 75 and 83% in manure from cows provided mineral blocks with 0.05 and 0.1% respectively (Barker and Jones 1976). Further laboratory trials recorded 99% mortality of face fly larvae *Musca autumnalis* De Geer (Diptera: Muscidae) provided with manure from cattle fed a ration containing $<$0.5 mg/kg diflubenzuron (Miller 1974).

Dimilin 2L® (22% diflubenzeron, Chemtura Corporation, Middlebury, CT), is a diflubenzuron formulation used in pastures and rangelands in the United States to suppress arthropod pests, such as the differential grasshopper, *Melanoplus differentialis* (Thomas), (Orthoptera: Acrididae) (Amarasekare and Edelson 2004) and the beet armyworm, *Spodoptera exigua* ((Hübner) (Lepidoptera: Noctuidae) (Decombel et al. 2004), while in other countries, such as Denmark, it is labeled for suppressing house fly populations associated with confined animal facilities (Kristensen and Jespersen 2003).

For most insecticide treatments for grasshoppers or armyworms in pastures, cattle are not allowed to return for a set time period after treatment to prevent the animals from being poisoned. However, this is not the case when using Dimilin 2L with cattle returned to pastures immediately after treatment. Currently, it is not known if treating pastures with Dimilin 2L® and allowing cattle to graze on treated forages will suppress horn flies development in resulting cattle manure. The objectives of this study were to: 1) determine if manure collected from cattle feeding on pastures treated with Dimilin 2L resulted in larval horn fly mortality, and 2) if suppression occurs, the duration of horn fly reduction.

Materials and Methods

Sites in Stephenville, Dublin, and Belton, TX were used in this study. At treated sites in Stephenville and Dublin, Dimilin 2L® was applied aerially at 59 ml/hectare. The treatment site in Belton received the same amount but by tractor with a 9.1 m boom sprayer containing nine nozzles spraying at a rate of 215 L solution/hectare. Aerial sprays were applied at a rate of 22.7 L/0.4 hectare. The control sites received no treatments. The treated sites were as follow: 1) Stephenville, 20.2 hectare and 26 cows with calves; 2) Dublin, 24.3 hectares and 20 cows; 3) Belton, 18.2 hectares and 25 cows. The control sites were as follow: 1) Stephenville, 11.3 hectares and 27 cows and 15 calves; 2) Dublin, 16.1 hectares and 20 cows; 3) Belton, 101.1 hectares and 88 cows. Treatments during year one were applied in Belton on 24 May 2004 and in Stephenville and Dublin on 1 June 2004. A second replication of the study was conducted in 2005 during the same time of year with similar cow numbers.

Three manure samples were taken from each treated and control site at a set interval. Initial manure samples were taken 3 d post treatment. Samples were then taken weekly for four weeks for a total of five sample periods. In order to collect samples, adult cows were monitored for deposition of fecal matter with each sample taken from a manure pat deposited from a different adult cow. Once deposited, approximately 500 ml of each fresh manure pat was placed individually in a 500 ml plastic Solo container, covered with a lid, and stored temporarily in a freezer set at 0°C at the Texas Agriculture Experiment Station (TAES) in Stephenville, TX. Samples were eventually transferred and stored in a freezer set at 0°C at the USDA-ARS Kerrville Research Facility, Kerrville, TX.

In order to determine the effects of Dimilin 2L on horn fly development, the following procedures were implemented. The oldest manure samples were removed from the freezer and allowed to thaw approximately 24 h prior to inoculation with horn fly eggs. Samples typically remained in the freezer for six to eight weeks prior to being examined. For each manure sample, 100 g was removed and placed in a 500 ml drinking cup. One hundred horn fly eggs were taken from a colony maintained at the Kerrville facility and placed on a 2.54 cm diameter filter paper. Filter papers with eggs were then placed on the surface of the manure in each cup and covered with a paper towel held in place with a rubber band. Cups were maintained in a growth chamber set at 27°C and 16L:8D photoperiod. A filter paper with 100 horn fly eggs was placed in a petri dish and stored in the rearing chamber to determine percent hatch. Numbers recorded for each sample were adjusted based on percent mortality recorded for the control horn fly egg sample.

Manure samples were sorted and horn fly pupae removed seven days after inoculation. Number of pupae and deformed pupae per sample was recorded for each sample. Deformed pupae were defined as dumbbell shaped, collapsed, or cigar shaped, while healthy pupae were cylindrical. For each sample, pupae were placed in a petri dish lined with a filter paper labeled with sample collection information. Petri dishes with pupae were covered and returned to the growth chamber. Percent adult emergence for each sample and percent normal and deformed pupae were recorded approximately 10–14 d after being placed in petri dishes. Data from each location were combined and analyzed using Proc ANOVA (SAS Institute 1998). Least significant difference (LSD) test was used following a significant F test ($P < 0.05$) to separate means (SAS Institute 1998). Data presented as percent of response were arcsine square root transformed prior to analysis (SAS Institute 1998).

Results

Data for percent survivorship of horn flies reared on manure from treated and control pastures (Figure 1) were examined. No overall significant difference ($F = 2.86$; df = 1, 39.3; $P = 0.0990$) between treated and control pastures was determined for percent survival of horn flies to the adult stage. However, a significant difference ($F = 24.66$; df = 4, 40.5; $P < 0.0001$) for date was determined. Furthermore, an interaction effect ($F = 5.54$; df = 4, 40.6; $P = 0.0012$) was determined between treatment and sample date. Site was also determined to be significant ($F = 3.91$; df = 2, 35.5; $P = 0.0293$) for survivorship to the adult stage. In contrast, year was not determined to be significantly

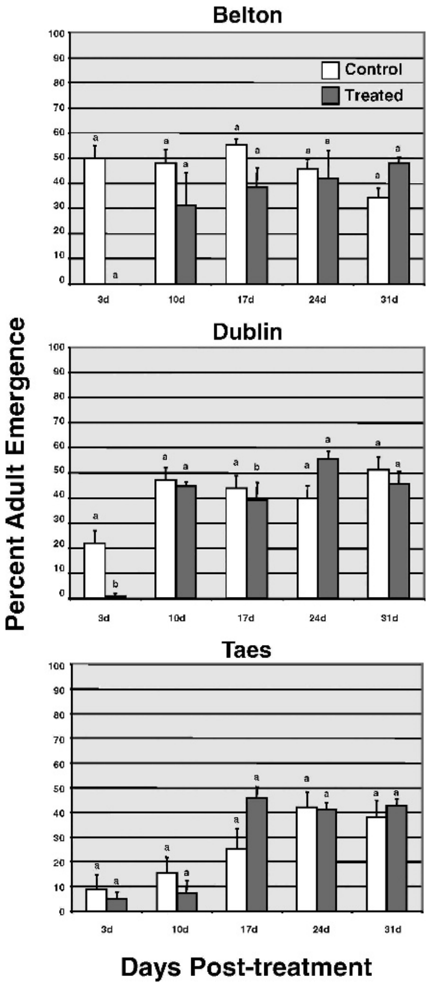

Fig. 1. Percent horn fly adult emergence ±SE when reared on manure sampled three sites treated and untreated with Dimilin 2L®. Means in a column followed by different letters are significantly different ($P \leq 0.05$; LSD, SAS Institute 1998). Values in columns not followed by capital letters were not significantly different across treatments. Percentage data were arcsine transformed prior to analysis.

different ($F = 2.31$; df = 1, 161; $P < 0.1308$). Therefore, data for each year were combined and results are presented in Figure 1. Significantly lower adult emergence was observed in the treated pastures in Dublin at 3 and 17 d post treatment when compared to the untreated control pastures (3d: $F = 80.6$; df = 1, 2; $P < 0.0001$ and 17d: $F = 11.79$; df = 1, 2; $P = 0.0089$).

Data for percent deformed pupae of horn flies reared on manure from treated and control pastures (Figure 2) were also examined. No overall significant difference for percent deformed pupae resulting from horn flies reared on manure

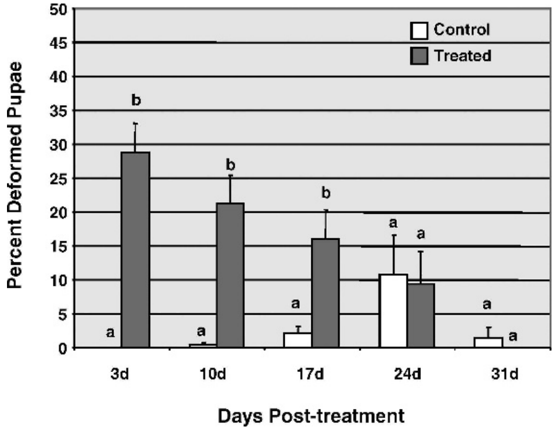

Fig. 2. Percent horn fly pupae deformed ±SE when reared on manure sampled from Dimilin 2L® treated and untreated pastures over time. Means in a column followed by different letters are significantly different ($P \leq 0.05$; LSD, SAS Institute 1998). Values in columns not followed by capital letters were not significantly different across treatments. Percentage data were arcsine transformed prior to analysis.

from treated and control pastures was determined. However, percent deformed pupae by sample date was significantly different. An interaction effect between treatment and date was also recorded. No significant difference in percent deformed pupae by site or year was recorded. Therefore, year and date data were combined and results are presented in Figure 2. Significantly more deformed pupae were observed in the treated pastures at 3, 10, and 17d post treatment when compared to the untreated control pastures (3d: $F = 41.79$; df = 1, 2; $P < 0.0001$; 10d: $F = 27.85$; df = 1, 2; $P < 0.0001$; 17d: $F = 9.48$; df = 1, 2; $P = .0042$).

Discussion

Dimilin 2L is an effective compound for the suppression of pasture and rangeland pests. Additionally, livestock can be returned to sites immediately after treatment. Animals can feed on treated sites without concerns of negative effects due to consumed diflubenzuron passing through the animals and being expelled in their solid wastes. However, an added benefit is that horn flies colonizing resulting wastes are affected.

Results for percent emergence were highly variable across year and site. This effect can be explained in part due to differences between sites. Each site varied in size and the number of cattle per hectare. Such a difference can result in the forage being consumed at much greater rates at one site versus the others. Additionally, consistency in application methodology (i.e. aerial versus tractor) could have resulted in insufficient coverage for some of the sites.

The effect of Dimilin 2L might be better demonstrated in the F_1 generation produced by those individuals to successfully emerge. House flies and stable flies treated topically with a 25% WP difulbenzuron formulation resulted in a 71 and 100% reduction in egg hatch respectively (Wright and Spates 1976). Adult horn flies housed in cages treated with 1 to 10% diflubenzuron dust resulted in 92 to 89% reduction in hatch of resulting eggs respectively (Kunz and Bay 1977).

The effects of Dimilin 2L on horn fly develop were more apparent when examining percent pupal deformation. Significant levels of percent deformation were recorded for samples taken up to 17 d post treatment. Therefore, although no difference in emergence was recorded between horn flies reared on manure from treated and control sites, a significant impact on the target horn fly population might be present due to reduced mobility and egg hatch from resulting adults.

Although studies examining the use of diflubenzuron for suppressing the horn fly date to the 1970s, we are not aware of it being labeled for such use until Chemtura Corporation added this information to its Dimilin 2L label in 2005. The reason why such a delay occurred between the publishing of Kunz and Bay (1977) and the many other studies on the use of this compound as a feed through and being marketed for horn fly control is not known. Regardless, its use for grasshopper control today can result in indirect benefits due to its impact on horn fly populations associated with livestock placed on these treated sites.

Horn flies represent only one of many species involved in the food web with cattle manure as its foundation. Other insects, such as dung beetles (Coleoptera: Scarabaeidae) depend on the manure as resource for themselves as well as their offspring. Additionally, the parasitic wasp *Muscidifurax raptor*, Girault and Saunders (Hymenoptera: Pteromalidae), as well as many others will target and colonize specific dipteran species commonly associated with the manure. Few data are available on the effects of diflubenzuron on these other arthropods. Dimilin appears to have no effect on the emergence of *M. raptor* or its ability to parasitize other hosts (Ables et al. 1975), but the effects on dung beetles are not known. Future studies need to examine more closely the effects of diflubenzuron on the fecundity of horn flies that successfully pupate in treated manure, as well as, its effects on other biological control agents including dung beetles.

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