ABSTRACT  Hay mixed with manure and urine residues at sites where hay has been provided as supplemental winter feed for cattle provide an excellent substrate for the development of immature stable flies, *Stomoxys calcitrans* (L.). Such sites are primary sources of early summer stable flies in the central United States and no effective measures are currently available to control fly development in them. A single application of granular cyromazine in May provided 97% reduction in the number of adult stable flies emerging from hay feeding sites. Stable fly control did not decline during the 12 wk season. A small decline in control was observed relative to anthomyiid, sarcophagid, and syrphid flies developing in the hay feeding sites. However, none of those flies are considered to be pests and ≥50% control of those flies was maintained for 65 d after application. Cyromazine offers a safe and affordable option for the control of immature stable flies developing in winter hay feeding sites. Controlling those flies should reduce the estimated $2 billion per year of lost production in U.S. cattle industries attributable to stable flies.

KEY WORDS  *Stomoxys calcitrans*, insect growth regulator, larval development

Stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), are important pests of livestock throughout much of the world. Immature stable flies develop in decomposing vegetative materials and often are associated with barn yard accumulations of manure. Over the past 30 yr, cattle producers in the central United States have adopted the practice of using fixed feeders to provide large round bales of hay to their pastured cattle during the winter (Broce et al. 2005). Immature stable flies readily develop in the accumulated hay and manure residues associated with winter hay feeding sites and such sites are considered to be primary sources of stable flies during early summer in the central United States (Broce et al. 2005; Talley et al. 2009, Taylor and Berkebile 2011). The practice of feeding cattle large round bales with stationary feeders has effectively extended the barn yard habitat into pastures resulting in increasingly frequent observations of stable flies infesting pastured cattle (Hall et al. 1982, Campbell et al. 2001, Broce et al. 2005). Pastured cattle respond more negatively to stable fly infestations than confined cattle (Campbell et al. 1987, 2001; Taylor et al. 2012) and one-half of the estimated $2 billion annual production losses attributed to stable flies in the United States are the result of their effects on pastured cattle (Taylor et al. 2012).

Producers in the central United States typically start providing cattle with hay in large round bale feeders to supplement forage in November and December each year (Broce et al. 2005). Feeders are placed in locations with convenient winter access and often are not moved during the feeding season. As animals congregate at the feeders, urine and manure combine with waste hay to make an ideal substrate for stable fly development (Talley et al. 2009). Accumulations can exceed 25–50 cm in depth (Broce et al. 2005; Talley et al. 2009; D.B.T., unpublished data) and extend 4–7 m from the feeder. Feeding sites are abandoned when forage becomes available in the spring. As the residues thaw and reach the appropriate stages of decomposition in the spring, they are colonized by stable flies. In eastern Nebraska, stable fly adults emerge from hay feeding sites from early May to late October; however most, over 90%, emerge during an 8-wk period from early June to mid-July (Taylor and Berkebile 2011). Adult emergence levels of 1,600–4,000 per m² have been reported (Broce et al. 2005, Taylor and Berkebile 2011). Because hay feeding does not begin until late fall, after adult stable fly populations have dropped to very low levels, it is unlikely that flies overwinter in them.

Methods for controlling immature stable flies in winter hay feeding sites have been elusive (Broce et al. 2005, Foil and Younger 2006, Hogsette et al. 2008). The preferred option, removal of residues, is expensive and labor intensive. Often, winter hay feeding sites are located in remote pastures and inaccessible...
for large equipment or repeated treatments. Hay feeding site residues have active microbial communities (Talley et al. 2009) capable of rapidly degrading most insecticides necessitating repeated treatments that are inconvenient and expensive. As a result, most hay feeding sites are left to naturally decompose, although providing developmental habitat for stable flies (Broce et al. 2005).

Cyromazine (N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine) is an insect growth regulator (IGR) that interferes with molting. Cyromazine is stable to hydrolysis and photolysis (USEPA 2006) and can remain active for 10–20 wk when applied to chicken manure for house fly (Musca domestica L.) control (Mulla and Axelrod 1983). The purpose of this study was to evaluate the efficacy of a single treatment of cyromazine to provide season long reduction in the number of stable flies emerging from winter hay feeding sites in eastern Nebraska.

**Materials and Methods**

The study was conducted in 2011 at the University of Nebraska, Agricultural Research and Development Center (ARDC) located near Ithaca, NE. Approximately 400 cattle were maintained on pasture through the winter with supplemental forage provided in large round bale feeders. Six hay feeding sites were identified, four at an area referred to as the round bale feeders. Six hay feeding sites were identified with supplemental forage provided in large equipment or repeated treatments. Hay feeding site residues have active microbial communities (Talley et al. 2009) capable of rapidly degrading most insecticides necessitating repeated treatments that are inconvenient and expensive. As a result, most hay feeding sites are left to naturally decompose, although providing developmental habitat for stable flies (Broce et al. 2005).

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Temperature and precipitation for the experimental period were obtained from the High Plains Regional Climate Center (University of Nebraska, Lincoln, NE) MEADAGROFARM station, located approximately in the center of the ARDC property (41.17° N, 96.47° W).

**Statistical Analysis.** Preliminary analysis indicated that the emergence trap collections were overdispersed. We therefore opted to use negative binomial regression analysis (Proc Genmod; SAS Institute 2008) to characterize effects of cyromazine on trap catches. In the first analysis, all flies collected were included. Treatment and species (stable fly and "other" flies) were treated as categorical variables. First and second order terms for days after treatment (D and D²) were considered continuous characters. Multiple collections from traps in the same location were considered to be repeated measures with an AR(1) covariance structure. Only stable flies were included in the second analysis. Treatment and sex were considered categorical variables and D and D² were continuous variables. Deviations from the 1:1 ratio of counts from treated and untreated zones expected under the null hypothesis that cyromazine had no effect upon emergence trap catches were evaluated with chi-squared analyses.

To assess changes in the level of control relative to time after treatment, hay feeding sites were considered replicates and trap catches were pooled by site and week after treatment. Control was calculated as 

\[ c = 1 - \left( \frac{n_t}{n_c} \right) \]

where \( n_t \) is the total number of flies captured in treated zone traps and \( n_c \) is the number of flies collected in untreated zone traps. Site-week combinations with \( n_c < 10 \) were excluded from the analysis. Variance of \( c \) was normalized with an arcsine-square-root transformation. Results are reported in their original units. Loss of control was evaluated by

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**Table 1. Number of flies collected in emergence traps located on cyromazine treated (25 g Neporex 2SG m⁻², 0.5 g AI m⁻²) and untreated zones of six winter hay feeding sites**

<table>
<thead>
<tr>
<th>Feeding site</th>
<th>Area (m²)</th>
<th>Stable flies</th>
<th>Other flies</th>
<th>Total % control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cyromazine</td>
<td>Un-treated</td>
<td>( \chi^2 )</td>
</tr>
<tr>
<td>B1</td>
<td>47</td>
<td>34</td>
<td>877</td>
<td>780.1</td>
</tr>
<tr>
<td>B2</td>
<td>31</td>
<td>65</td>
<td>319</td>
<td>168.0</td>
</tr>
<tr>
<td>B3</td>
<td>69</td>
<td>129</td>
<td>244</td>
<td>35.5</td>
</tr>
<tr>
<td>B4</td>
<td>24</td>
<td>3</td>
<td>106</td>
<td>97.3</td>
</tr>
<tr>
<td>T1</td>
<td>64</td>
<td>13</td>
<td>938</td>
<td>899.7</td>
</tr>
<tr>
<td>T2</td>
<td>61</td>
<td>20</td>
<td>1,196</td>
<td>1,139.3</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>264</td>
<td>3,682</td>
<td>2,960.6</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>54</td>
<td></td>
<td>96.6%</td>
</tr>
</tbody>
</table>

\( df = 1; P < 0.01 \)
regressing \( c \) against \( D \) and \( D^2 \) (Proc Reg; SAS Institute 2008).

Temporal differences in emergence patterns were evaluated by comparing empirical distributions of emergence trap collections with the Kolmogorov–Smirnov test (Proc Univariate; SAS Institute 2008). Alpha = 0.05 was used for all analyses.

**Results**

Stable flies were the most frequently collected brachycerate flies in the emergence traps representing 64% of the 6,186 collected. Several additional species were collected emerging from the hay feeding sites, the most common of which were *Syrattia pipiens* (L.) (Syrphidae), *Scathophaga stercoraria* (L.) (Scathophaoidae), *Adia cinerella* Fallen (Anthomyiidae), and *Eristalis tenax* (L.) (Syrphidae). Nonstable fly brachyerate Diptera were pooled as “other flies” for analysis. Emergence trap collections in untreated zones averaged 2.6 stable flies and 1.2 other flies per trap day \( (t^{-1} \text{d}^{-1}) \). In cyromazine treated zones, an average of 0.2 stable flies and 0.4 other flies were collected per trap day (Fig. 1).

No fly larvae were observed in the hay circle substrate on 11 May, the day of treatment. During the first sampling period, 24–27 May, only one stable fly was collected. However, 259 other flies, primarily *Scathophaga stercoraria*, were collected, 61 in treated zones and 198 in untreated zones. Stable fly emergence in untreated zones exceeded a median of \( > 1 \) fly \( t^{-1} \text{d}^{-1} \) during the 2–6 June sampling period and peaked during the next period, 6–9 June, at 16.7 flies \( t^{-1} \text{d}^{-1} \). Untreated zone collections declined to 2–3 flies \( t^{-1} \text{d}^{-1} \) after 14 June, where they remained until 26 July when they further declined to \( < 0.3 \) flies \( t^{-1} \text{d}^{-1} \). Most of the flies collected after the decline in stable fly emergence were *S. pipiens*.

Cyromazine reduced the number of emerging flies by 87% (Table 1; \( \chi^2 = 34.1; \text{df} = 1, P < 0.01 \)), but was more effective against stable flies than other flies, 97 and 64% reductions, respectively (\( \chi^2 = 6.0; \text{df} = 1, P = 0.01 \)). The efficacy of cyromazine treatments varied among hay circles from 60 to 93% (Table 1; \( \chi^2 = 24.0; \text{df} = 5, P < 0.01 \)). The number of flies collected emerging from untreated zones declined from a peak of 18.9 flies \( t^{-1} \text{d}^{-1} \) in early June to 0.5 flies \( t^{-1} \text{d}^{-1} \) in late July. The decline was correlated with \( d \) after treatment squared (\( D^2; \chi^2 = 6.0; \text{df} = 1, P < 0.01 \)). The three-way interaction between species, treatment, and \( D^2 \) was significant as well (\( \chi^2 = 9.8; \text{df} = 3, P = 0.02 \)). The significant interactions between species and treatment observed in the previous analyses indicated that stable flies and other flies differ in their phenology and response to cyromazine. Therefore, separate analyses were conducted on the two groups. Cyromazine treatment reduced stable fly emergence by 97% (median of six sites, \( \chi^2 = 25.8; \text{df} = 1, P < 0.01 \)), but the effect was not equal with respect to males and females (\( \chi^2 = 12.0; \text{df} = 1, P < 0.01 \)). Numbers of males and females were approximately equal in untreated zones (1,905 \( \varnothing \) and 1,777 \( \delta \)); however, females out numbered males by nearly a 3:1 ratio in cyromazine treated zones (Fig. 2; 197 \( \varnothing \) and 67 \( \delta \)). Efficacy of the cyromazine treatments varied among hay circles (\( \chi^2 = 11.3; \text{df} = 5, P = 0.05 \)). Stable fly emergence was reduced by 99% in site T1, but only 47% in site B3. Two successive collections representing 14–20 June, from two traps in the treated zone of B3 captured 104 stable flies, nearly 40% of the 264 collected in treated zones during the study. Stable fly collections in untreated zones declined from their peak on 9 June relative to \( d \) after treatment squared (\( D^2; \chi^2 = 5.5; \text{df} = 1, P = 0.02 \)). Treatment effects did not vary in a linear (\( \chi^2 = 0.2; \text{df} = 1, P = 0.69 \)) or curvilinear (\( \chi^2 = 0.4; \text{df} = 1, P = 0.54 \)) manner relative to \( d \) after treatment. No significant linear or curvilinear relationship between control of stable flies and \( d \) after treatment was ob-
Density, species composition, and phenology of flies collected emerging from the untreated zones of the hay feeding sites during this study were similar to those observed in previous studies (Broce et al. 2005, Talley et al. 2009, Taylor and Berkebile 2011) indicating that the hay feeding sites were representative of those observed in the central United States. Temperatures were near normal and precipitation was higher than normal, especially during the 6 wk after application. Heavy precipitation can leach cyromazine from the substrate (Pote et al. 1994) reducing its residual effects. Longer residual effects may be seen in years with less precipitation.

A single treatment of 0.5 g cyromazine m$^{-2}$ in mid-May, reduced the number of stable flies emerging from winter hay feeding sites by over 95%. Control was maintained throughout the expected season for stable fly development, June–August (Taylor and Berkebile 2011). No discernable decline in stable fly control was observed up to 12 wk after cyromazine application. Emergence trap collections in untreated zones were too low to evaluate control beyond 12 wk. In previous studies, cyromazine sprayed on chicken manure suppressed house fly emergence for 9 wk (Mulla and Axelrod 1983) and feed-through applications suppressed emergence for 20 wk (Brake et al. 1991). The marginally significant decline in control observed with respect to other fly species (none of which are considered pests) maintained ≥50% control for 65 d post treatment.

The suitability of the winter hay feeding site substrate for stable fly larval development is dependent upon its state of decomposition. Stable flies prefer “aged” substrate with an active microbial community (Broce and Haas 1999, Talley et al. 2009). In eastern Nebraska, hay feeding site substrates are suitable for stable fly development for ∼5 wk, from mid-May through mid-July (Taylor and Berkebile 2011). One concern is that suppression of fly development in substrates may delay decomposition (Madsen et al. 1990) such that they may remain suitable for fly development after the control agent has degraded. Visually, no differences in the state of decomposition of substrates in treated and untreated zones were apparent. Although peak stable fly emergence from treated zones occurred ∼2 wk after that for untreated zones (Fig. 3), no indication of extended suitability of treated zone substrate for fly development or adult emergence was observed.

Cyromazine was slightly less effective for controlling brachycerous Diptera other than stable flies emerging from the hay feeding sites by 64% ($\chi^2 = 11.0; df = 1, P < 0.01$) and the level of reduction did not differ among sites ($\chi^2 = 9.4; df = 5, P = 0.09$). The number of other flies collected was highest in the first collection period, 24–27 May, with 3.7 flies t$^{-1}$d$^{-1}$ and decreased relative to D ($\chi^2 = 27.3; df = 1, P < 0.01$). The cyromazine treatment effect varied relative to D ($\chi^2 = 4.2; df = 1, P = 0.04$) and a tendency toward decreasing control relative to D$^2$ (Fig. 1; $c = \sin(0.98 - 4.5 \times 10^{-5} \times D^2); F = 5.4, df = 1.63, P = 0.06; r^2 = 0.05$) was observed. According to this relationship, cyromazine maintained 50% control of other flies for 65 d after application. Other flies emerged from the treated zones later than they did from the untreated zones (Fig. 3; $D = 0.13, KS_{n} = 2.35, P < 0.01$).

Precipitation during the study was heavy. During the 24-h period after application, 6.3 cm of rain was recorded. Run-off was severe and washed away areas of feeding site residue. Total precipitation for May after treatment, 13.9 cm, was double normal (1,970–2,000 average) and 37% more rain than normal was recorded in June. Temperatures were near normal during the study.
the cyromazine treatment was effective within 2 wk of application and that it may be possible to delay application for 2–3 wk extending fly control further into late summer, while retaining control in early June.

Cyromazine has been used widely for controlling flies associated with livestock, especially house flies (Hall and Foehse 1980, Axtell and Edwards 1983, Mulla and Axelrod 1983, Kocisova et al. 2004). House fly resistance to cyromazine, although not widespread, has been reported in Europe, North, and South America (Bloomcamp et al. 1987, Kristensen et al. 2001, Pinto and do Prado 2001, Acevedo et al. 2009, Bell et al. 2010). House flies do not use hay feeding site substrates for larval development (Taylor and Berkebile 2011) and none were collected emerging from the sites during this study. Stable fly resistance to cyromazine has not been reported.

Nearly 58% of the stable flies collected emerging from treated zones during the 12 wk of this study were collected during two successive collections representing 14–20 June. Corresponding collections from untreated zones represented only 5% of their season total. Site B3 accounted for a large proportion of those flies with 104 stable flies from the treated zone being recorded during that period. Those flies represent 40% of the stable flies collected emerging from all of the treated zones during the study and 80% of those from site B3. All but one of the stable flies collected emerging from the treated zone of B3 during that time period were collected in two traps. Stable fly control at site B3 was only 47%, whereas mean control for the other five sites was 94%. Control at site B3 excluding flies collected between 14 and 20 June was 88%. Reasons for the relatively high numbers of stable flies emerging from treated zones between 14 and 20 June and especially from site B3 are not clear. Possible explanations are 1) the two traps with all but one of the treated zone flies were placed on debris washed into site B3 from a nearby, untreated, hay circle by the heavy rains; 2) uneven dispersal of the Neporex resulted in inadequately treated areas within the treated zone; or 3) cyromazine retarded fly development and those emerging from the treated zones 14–20 June were remnants of the same cohort that produced peak emergence in the untreated zones 1 wk earlier (Fig. 1). A small percentage of survivors from the larger, earlier cohort, overlaid upon the much smaller succeeding one may be responsible for the observed emergence pattern. Further studies evaluating sublethal effects of cyromazine are needed to test this scenario.

Among stable flies collected emerging from hay feeding site substrates treated with cyromazine, females outnumbered males by a ratio of nearly 3:1. Sex ratio of stable flies emerging from untreated zones in this study, and from hay feeding site substrates in a previous study (Taylor and Berkebile 2011), approximated 1:1. We were unable to find reference to the sex ratio of survivors in previous studies on the effects of cyromazine on muscoid flies. Although the number of survivors was small, and we do not expect this bias to affect control efforts, it may be possible to use it as an indicator of treated populations. Applying this criterion to stable flies collected emerging from the treated zone of site B3 between 14 and 20 June, the sex ratio was 4.7:1 indicating that the substrate from which they had emerged was treated.

The granular formulation of cyromazine was more convenient and easier to apply than a liquid formulation. However, adequate moisture is needed to dissolve the granules once they have been applied. For liquid spray application, the Neporex 2SG label indicates dissolving in one gallon of water to cover 200 feet	extsuperscript{2}. This is the equivalent of 0.02 cm of precipitation. Average precipitation (2000–2011) for the second half of May at the study location was 5.2 cm (range, 1.4–9.9 cm). Thus, during even the driest year, 70 times more precipitation was received than the recommended amount of water for spray application. Furthermore, Talley et al. (2009) reported that hay feeding site substrates were 70% water at the time of peak stable fly emergence in June. In May, when the granules were applied, the substrate was soft with free-standing water in depressions and exuded when compressed. Under the conditions observed in eastern Nebraska, adequate moisture normally is available to dissolve the granules, both as moisture in the substrate and from precipitation. In situations where less precipitation is expected, or the substrate is drier, it may be necessary to follow label recommendations for spraying or wet the substrate after dry scattering cyromazine granules.

Stable flies developing in an average winter hay feeding site can reduce the productivity of nearby cattle by an estimated $150-$1,000 depending upon their production sector (Taylor et al. 2012). Treatment of such a site with cyromazine costs approximately $10 and requires minimal labor resulting in a net return-to-cost ratio of between 10 and >50:1. IGRs have low levels of vertebrate toxicity because their target systems are restricted to insects and other arthropods (Tunaz and Uygun 2004). The acute LD	extsubscript{50} for cyromazine is >3,000 mg/kg (rat) and no effects on human health have been reported (Bostanian 2004). Used according to label, cyromazine offers a safe, effective, and economical control option for stable flies developing in winter hay feeding sites.

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