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Protocols

Methods for Surveying Stable Fly Populations

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Abstract

Stable flies are among the most important pests of livestock throughout much of the world. Their painful bites induce costly behavioral and physiological stress responses and reduce productivity. Stable flies are anthropogenic and their population dynamics vary depending on agricultural and animal husbandry practices. Standardized sampling methods are needed to better identify the factors controlling stable fly populations, test novel control technologies, and determine optimal management strategies. The current study reviewed methods used for a long-term study of stable fly population dynamics in the central Great Plains. An additional study compared the relative size of flies sampled from the general population with that of flies sampled emerging from substrates associated with livestock production. Flies developing in livestock associated substrates are significantly larger than those in the general population indicating that other types of developmental sites are contributing significant numbers of flies to the general population. Because efforts to identify those sites have yet to be successful, we speculate that they may be sites with low densities of developing stable flies, but covering large areas such as croplands and grasslands. The stable fly surveillance methods discussed can be used and further improved for monitoring stable fly populations for research and management programs.

Key words: Stomoxys, trap, population dynamics

Stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), are synanthropic, hematophagus pests of livestock, wildlife, and humans worldwide. Despite years of effort, several aspects of stable fly population dynamics remain unsettled including dispersal, overwintering, and phenology. The primary factor controlling stable fly populations appears to be the availability of suitable developmental substrates. Most of the characterized developmental substrates are anthropogenic (Cook et al. 2018). In addition, adult dispersal is primarily dependent on host availability (Bailey et al. 1973), and most of the hosts are livestock or companion animals, which are dependent on human activities. Human agronomic and animal husbandry practices have changed over time and differ among regions of the world, even within countries; therefore, the fundamental drivers of stable fly population dynamics vary both historically and spatially. Stable flies are continuously adapting to exploit novel agronomic and animal husbandry systems. These confounding factors contribute to a lack of consensus regarding fundamental aspects of stable fly biology over time and from place to place. Several of the unresolved questions related to stable fly population dynamics revolve around a lack of concordance between independent assessments of immature (larval) and adult populations. Contemporaneous sampling of both life stages is needed to resolve this issue. Standardized sampling and quantitative methods are needed to compare stable fly population dynamics among disparate agronomic practices, environments, and eras.

Systematic sampling can be used to investigate many aspects of stable fly (and insect in general) biology including intra- and interspecific diversity (Szalanski et al. 1996, Masmeatathip et al. 2006, Lorn et al. 2020), population dynamics and phenology (Mihok et al. 1996, Guo et al. 1998, Heath 2002, Rodríguez-Batista et al. 2005, Pitzer et al. 2011, Beresford and Sutcliffe 2012, Urech et al. 2012, Jacquiet et al. 2014, Solórzano et al. 2015, Godwin et al. 2018, Lendzele et al. 2019), vagility and dispersal (Gersabeck and Merritt 1985, Hogsette and Ruff 1985, Taylor et al. 2010), and behavior (Broce et al. 1991, Beresford and Sutcliffe 2008).

Most traps for adult stable flies rely on visual cues and are based on either the sticky trap design of Williams (1973) or the blue-black fabric traps developed for tsetse fly (*Glossina* spp., Diptera: Glossinidae) management (Mihok et al. 1995). The Williams trap consisted of two pieces (35 × 45 cm) of translucent Alsynite fiberglass interlocked at the middle to form four vanes. Vanes were either covered directly with Tack Trap or covered with a plastic sleeve before applying the Tack Trap. Broce (1988) modified the Williams trap by using a single piece (30 × 60 cm) of Alsynite formed into a cylinder. The Broce traps are easier to construct and less expensive than the Williams trap. In addition, because they can be covered with a single plastic sleeve rather than the four required for a Williams

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trap, they are easier to maintain. Alsynite fiberglass panels have become difficult to obtain over the last 20 yr. In response, Beresford and Sutcliffe (2006) examined alternate materials and found white Coroplast panels to be better than Alsynite for attracting stable flies, and their attractiveness was more stable in the field.

Many permutations of blue-black fabric traps have been proposed and tested for collecting both stable flies and tsetse flies (Brightwell et al. 1987, 1991; Laveissière and Grébaut 1990, Kappmeier 2000, Mihok 2002). In the tropical environment on Reunion Island, Gilles et al. (2007) found the Vavoua trap to be more efficient than Broce sticky traps and recommended their use for the control of stable flies over other blue-black cloth traps because of their ease of use. In Costa Rica, although sticky traps were more efficient than blue-black cloth traps, Solórzano et al. (2015) recommended Vavoua traps because they were easier to maintain and more environmentally friendly.

The addition of olfactory cues to either sticky traps or blue-black cloth traps can increase catch (Cilek 1999, Zhu et al. 2016). However, for surveillance programs, we have not found odorants to be necessary. They do show promise for increasing trap catch for control programs.

Trap choice for a stable fly surveillance program is dependent on the goals, scope, and duration of the project. All traps have biases, whether relative to sex, age, or physiological state and those biases must be considered when interpreting trapping data. When measuring relative population levels, trap efficiency is not necessarily advantageous as long as enough flies are collected to measure the desired parameters. Trap efficiency will be of greater concern in mark–release–recapture studies or when population management is a program goal. We have used both Alsynite cylinder traps and Coroplast panel traps with similar success in our research and surveillance programs. Under the environmental conditions experienced in the Great Plains of the United States, we have not found blue-black cloth traps to be suitable for extended surveillance programs. Frequent high winds reduce the efficacy of free hanging Vavoua traps (DBT, unpublished observations) and grasshoppers (Orthoptera: Acrididae) chew the mesh portions of the traps creating holes through which flies can escape (Taylor and Berkebile 2006).

We used Broce's modification of the Williams trap for our long-term survey of stable fly populations (Taylor and Berkebile 2006). These studies were initiated prior to Coroplast being recognized as a suitable substitute and the Broce traps were retained to maintain continuity in the dataset. Because stable flies fly low to the ground (Williams and Rogers 1976, Gersabeck and Merritt 1983), traps should be mounted as low as possible while maintaining visibility above vegetation (Beresford and Sutcliffe 2008).

Adult stable fly populations can be quantified with on-animal counts as well. Standard leg counts have been used most often owing to the stable fly's preference for feeding on the lower legs of the host (Berry et al. 1983). Although leg counts are useful for assessing the immediate impact of stable flies on cattle, they can vary greatly depending on immediate environmental conditions and observer acuity. On-animal counts are very labor intensive, requiring at least one observer at each sampling site for the duration of the sampling period. Furthermore, in studies with restrained and penned animals, we observed significant numbers of stable flies feeding on the back of the pastern where they were not visible without very close observation (DBT, personal observation). Because studies of population dynamics require robust and consistent data sets from multiple sites, leg counts are not appropriate for monitoring stable fly populations across cultures, environments, and time although they can be useful for evaluating infestation levels at a given location and time.

Stable fly trap catch rates among trap locations can vary greatly in numbers and seasonal patterns even within a limited geographical range (Taylor et al. 2013). Multiple trap replicates are needed to assess stable fly population densities and demographics. Fifteen to 20 traps are needed to maintain the SEM catch within 30% of the mean for most of the stable fly season. Higher numbers of traps are needed to maintain the same standard when catch rates are lower at the beginning and end of the season. Trap catches are spatially aggregated with autocorrelation extending to ≈2 km. For enumerating a population over a landscape, traps should be separated by 1.5–2.0 km. Landscape features can affect trap catches on a local scale. Catch rates can differ by several fold on traps located within 1 km of each other (Taylor et al. 2013). Alsynite and Coroplast traps are most effective when placed in full sunlight (Agee and Patterson 1983). Shaded and enclosed sites should be avoided.

For quantitative sampling of immature stable flies, either core samples of developmental substrates (Berkebile et al. 1994, Talley et al. 2009) or emergence traps (Taylor and Berkebile 2011) have proven useful. Core samplers can be constructed from PVC tubing. However, because substrates typically contain fibrous vegetative materials such as straw, taking core samples can be challenging. A golf putting green hole cutter will cut through the fibrous materials better, but extracting a core can still be difficult, especially when substrates have high water content. An advantage of core samples is that the physical and biological properties of the substrate sample can be assessed and related directly to the presence or absence of immature flies (Talley et al. 2009, Wienhold and Taylor 2012, Friesen et al. 2016). Emergence traps sample a larger area than core samplers, thus reducing error due to the clumpy distribution of fly larvae within substrates. Emergence traps also eliminate the labor-intensive need to separate immature flies from the substrate for enumeration. Pupal traps are a third way to sample immature flies in developmental substrates (Hogsette and Butler 1981, Skoda et al. 1996). Pupal traps work on the principal that stable fly and house fly larvae seek a slightly drier substrate for pupariation. Traps are constructed out of ¼ inch mesh (6.35 mm) hardware cloth cylinders filled with wood shavings or other porous, substrate. Mature larvae pass through the hardware cloth and pupariate in the porous substrate. Traps are placed in the substrate in developmental sites and collected 5 d later. Our design for pupal traps differs from that presented by Hogsette and Butler (1981). Two pieces of 2-inch (5 cm) dia. PVC tube 2.5 cm long are wrapped with a piece of ¼-inch mesh hardware cloth (20 × 16 cm) and held in place with 2.5-inch (≈6.5 cm) ring clamps to create a 5 cm dia. × 20 cm long cylinder with PVC on each end. A 2-inch (5 cm) slip PVC plug is placed in one end of the cylinder. The cylinder is loosely filled with wood shavings and a plug placed in the other end. The traps are placed horizontally, just beneath the surface of the substrate in a fly development site and collected 5 d later. In the laboratory, the plugs are removed from the ends and the substrate emptied into a tray where the fly pupae can be isolated, enumerated, and processed as needed. Pupal traps are most useful in situations where livestock cannot be excluded from the study area. However, inability to recover 50% or more of the traps due to damage or loss is not uncommon (Skoda et al. 1996; DBT, unpublished observation).

Data on spatial variation and minimum number of traps are not available for immature fly sampling methods. Typically, we use six emergence traps on a hay circle ranging in size from 50 to 100 m^2. Emergence traps are relocated every 2 wk to avoid depletion of immatures in the sampling area because of exclusion of ovipositing females. Additional studies are needed on core sample and pupal trap variance to determine the optimal number of traps needed to achieve a given level of confidence.

Previous studies observed discordance between adult and immature stable fly population dynamics. In the central Great Plains, adult stable fly populations often exhibit a bimodal pattern with peaks in early and late summer and reduced numbers of flies during mid-summer (Taylor et al. 2007, 2017). Immature developmental sites coinciding with the early summer peak have been identified and characterized (Broce et al. 2005, Taylor and Berkebile 2011). However, developmental sites responsible for the late summer peak have been elusive.

Determining the relative contributions of different immature developmental sites to the general adult stable fly population is difficult. In order to begin addressing this issue, a system study combining adult and immature surveillance was conducted. Stable flies, like all holometabolous insects, do not grow or change size as adults. Adult size is determined by the quality of the immature developmental habitat (Florez-Cuadros et al. 2019). Therefore, adult size can be used as an indicator of the quality of the developmental habitat. Furthermore, if a given larval developmental habitat is the primary contributor to the adult population, then the size distribution of the population should parallel the size distribution of the flies emerging from that developmental habitat. The current manuscript discusses methods used for assessing stable fly populations, both adult and immature, and efforts to develop a system-based approach for understanding stable fly population dynamics.

Experimental Design

Experiments were conducted at the University of Nebraska, Eastern Nebraska Research and Extension Center (ENREC, formerly known as Agricultural Research and Development Center [ARDC]), Ithaca, NE. ENREC is approximately 4,000 hectares of crop and pasture land with ≈3,000 head of cattle located at 41.16 N, 96.46 W approximately 50 km NE of Lincoln, NE.

Twenty-five round Alsynite traps (Broce 1988) were placed in a grid pattern at each of the Public Land Survey System section corners. Traps were constructed by forming a 30.5 × 91.5 cm piece of flat Alsynite into a cylinder with ≈2.5 cm overlap. The overlapping portion of the Alsynite cylinder was inserted into a 35 cm slot in a 120 cm long 2”×2” lumber posts and secured with two 1½” drywall screws or 2” × 3/16” machine screws. The top of the slot was secured with a zip tie. Before attaching traps, posts were driven into the ground such that the bottom of the trap would be 60 cm above ground level. The Alsynite was covered with a 10-mil SurFlex plastic sleeve (Flex-o-glass, Inc., Chicago, IL) secured with four, 1” binder clips (Fig. 1). A paint brush was used to coat the sleeve with Tangle-Trap (The Tanglefoot Co., Grand Rapids, MI) diluted 1:1 with low-odor paint thinner (Sunnyside Corp., Wheeling, IL; Taylor et al. 2007).

During the active stable fly season (June–September), Alsynite traps were maintained twice per week by removing the plastic sleeve and replacing it with a fresh sleeve and applying fresh adhesive. Exposed sleeves, with flies, were returned to the laboratory for processing. During periods of relatively low-stable fly population densities, the frequency of traps maintenance was reduced to once per week to preserve resources. Depending on experimental goals, captured stable flies were counted, removed from traps, and stored in 20-ml scintillation vials. Flies in vials were either kept frozen or submerged in 80% ethanol. Flies preserved by either method can be sexed by the shape of the eyes or genitalia and their wings can be mounted on slides for size measurement. Frozen females can be dissected to characterize their physiological state (Scholl 1980).

Fig. 1. Dr. Broce examining a cylindrical Alsynite sticky trap.

Emergence traps were placed on suspected stable fly developmental substrates. Initially, emergence traps were constructed from 2”×4” lumber (Taylor et al. 2012). Subsequent traps were based on 5-gallon plastic buckets (Fig. 2A; Taylor et al. 2013). Bucket emergence traps were constructed by removing the bottom of the bucket and the center of the lid with a dry wall cutting bit (#560, Dremel, Racine, WI). The cutting edge for this bit does not extend to the tip; with the cutting depth properly set, the tip can be used to guide the bit around the bucket without cutting into the side. A semicircle (170°) was cut from 12 mesh/inch galvanized screen (r = 28 cm), and another circle was removed from the center of the screen (r = 2 cm; Fig. 2B). The screen was formed into a cone with 2 cm overlap, fitted into the hole in the lid of the bucket, and fused into place by heating the plastic of the lid with a heat gun and pressing the screen into the soft plastic. The top of the screen cone was trimmed to accept a 1¼” × 1” SPG × FPT bushing with ≈1 cm of screen overlap and held in place with a 1¾-inch hose clamp (Table 1). Pop rivets were used to secure the overlapping screen on the side to secure the cone. The collecting head was constructed by gluing a 6.5-cm-long piece of 1” PVC into a 1” slip × MPT adapter. A 2.5-cm square piece of aluminum window screen was formed into a cone and attached to the opposite end of the 1” PVC with hot glue. A hole was made in the tip of the screen cone with a sharpened pencil (7 mm dia.). A collecting chamber was constructed by drilling a 1¼” hole in the bottom of a 16 ounce ‘Twist & Store’ screw-top food storage container (Walmart, Bentonville, AR) with a hole-saw. The PVC assembly was inserted into the hole in the chamber and glued into place with hot glue. The MPT adapter of the collecting assembly was screwed into the FPT bushing on the top of the trap (Fig. 2C). Two collecting assemblies were made for each trap. Bucket emergence traps were installed

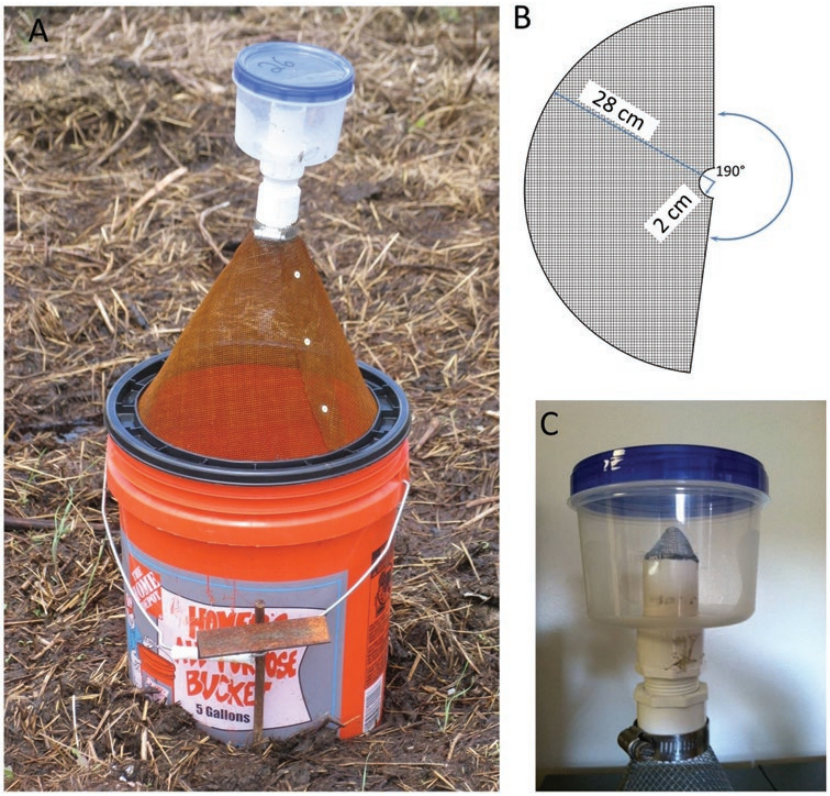

Fig. 2. (A) Complete bucket emergence trap. (B) A semicircle (170°) was cut from 12 mesh/inch galvanized screen (r = 28 cm) and a circle of screen was removed from the center (r = 2 cm) as well. (C) Collection assembly.

Table 1. Part list for bucket emergence traps

Part	Size	Quantity
Plastic bucket	5 gal. (≈20 l)	1
screen	12/inch mesh	30 × 30 cm
Pop rivets	1/8"	3
SPG × FPT bushing	1¼" × 1"	1
Ring clamp	1¾"	
PVC pipe	1"	6.5 cm
Slip × MPT adapter	1"	1
Aluminum window screen	16/inch mesh	3 × 3 cm
Food storage container	16 oz (≈500 ml)	1

by using a spade to cut a slit into the substrate and pressing the trap into it. In windy locations, a stake is driven next to the trap and hooked to the bail of the bucket to add stability. Traps were serviced weekly by removing the collecting head, placing a piece of cotton in the PVC tube to prevent the escape of flies, and a clean head installed. Emergence traps were relocated within their site every 2 wk to avoid depletion of emerging adults due to exclusion of ovipositing females by the traps. Used collecting heads were returned to the laboratory, frozen (–20°C), and the captured flies removed and processed.

To assess the size of adult stable flies, the length of the discal-medial (DM) cell of the wing was measured (Fig. 3). This metric was used because the DM cell is the longest cell of the stable fly wing making it the easiest to measure. In addition, it is located in the middle of the wing, so it is rarely damaged in wild caught or older flies. Many wings can be mounted on a glass microscope slide for measurement and maintained as permanent records without special storage needs. Wings were mounted using a drop of clear nail polish as the adhesive. The DM cell was measured with a digital microscope (Dino-lite Edge 5MP digital microscope, Torrance, CA) at ≈50× magnification (Florez-Cuadros et al. 2019). The length of the DM cell is related to the weight of the fly puparia by a cubic function (Florez-Cuadros et al. 2019).

Each time the ENREC sticky traps (Broce 1988) were sampled, a random sample of 100 flies was sexed, and wings were mounted on slides for measurement. Emergence traps were deployed as part of studies on stable fly larvicides. All adult flies collected from untreated (control) site emergence traps on what was believed to be the primary source of the flies at ENREC, circles where hay had been fed to the cattle during the previous winter, were processed similarly. Stable fly surveys of the ENREC facilities had detected very few immature stable flies outside of those observed in the hay feeding circles. The size distribution of the flies emerging from the winter hay feeding sites was compared with that of the flies collected on the Broce sticky traps. Collections from 2011 to 2018 were used for this study because they represented the years with the most complete sets of weekly collections from both types of traps.

Generalized linear mixed models (Proc GLIMMIX, SAS 9.4, Cary, NC) were used to evaluate the length of the DM cell relative to the trap type, emergence trap or sticky trap. Trap counts were summed within weeks. Week × year was included in the model as a random variable. Lengths of the DM cell were squared to improve the normality of the residuals.

Results

For the sticky traps, a total of 134,481 and 87,948 flies were collected in 2011 and 2018, respectively, of which 3,255 (42% ♀) and

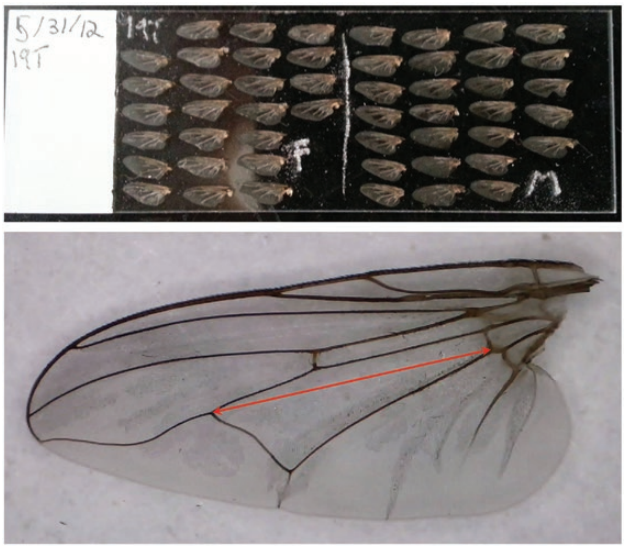

Fig. 3. Stable fly wings mounted on slide with clear finger nail polish (top) and an enlarged wing with the discal-medial cell measurement highlighted with red arrow (bottom).

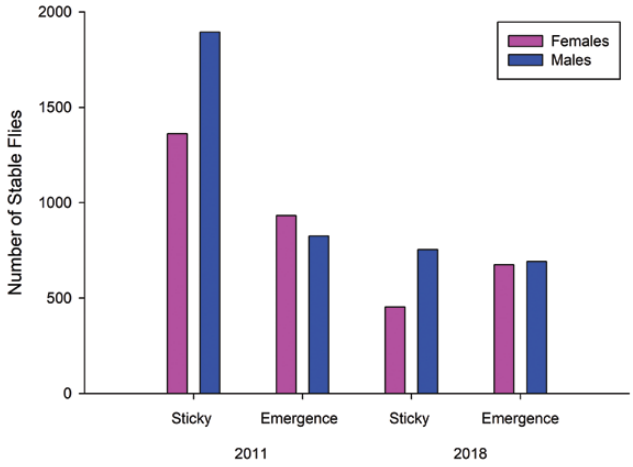

Fig. 4. Number and sex of stable flies sampled from sticky and emergence traps in 2011 and 2018.

1,207 (38% ♀) individuals were measured. For the emergence traps, 1,759 (53% ♀) and 1,365 (49% ♀) flies were collected and measured in 2011 and 2018, respectively (Fig. 4).

In 2011, the first fly was collected from a sticky trap on 28 March and the population peaked in the middle of June. Emergence trap collections began at the end of May and peaked at the end of June. The last fly was collected emerging from the hay circles on 28 July. A second collection peak was observed on the sticky traps in early October (Fig. 5).

In 2018, the first flies were collected from the sticky traps in early May and the collections peaked early to mid-June. The first flies were collected from the emergence traps in mid-May; emergence trap collections peaked in early to mid-June and dropped to low levels until the final fly was collected in early August. No second peak was observed on the sticky traps in 2018, although flies were collected on the sticky traps into November.

Stable flies collected in emergence traps placed on the hay feeding circles were significantly larger ($\bar{x}$ DM cell = 2.622 mm) than those collected on sticky traps $\bar{x}$ DM cell = 2.479; F = 413.38, df = 1, 7534, $P < 0.0001$). Female flies were larger $\bar{x}$ DM cell = 2.611 mm) than male flies $\bar{x}$ DM cell = 2.490; F = 882.55, df = 1, 7534, $P < 0.0001$), but no interaction between trap type and sex was observed (F = 1.34, df = 1, 7534, $P < 0.246$). Using the relationship between DM cell length and puparia weight developed by Florez-Cuadros et al. (2019; wt = $DM^{2.98}$), puparia of flies developing in the hay feeding circles were 18% heavier than those in the general population.

Discussion

The methods presented in this manuscript can be used to evaluate the phenology of stable flies relative to weather variables (Taylor et al. 2007, 2017) and immature fly population dynamics (Taylor and Berkebile 2011), dispersal (Taylor et al. 2010), and management (Taylor et al. 2012, 2014).

This study indicated that the size of flies emerging from the 'primary' immature developmental areas at the study site (Broce et al. 2005, Taylor and Berkebile 2011) was significantly larger than those in the general population. This implied that flies developing at other sites, with poorer developmental habitats, are contributing significantly to the general population. Unfortunately, the location and composition of those developmental substrates remain speculative. Mark–recapture studies conducted on ENREC found the median dispersal distance of stable flies to be ≈1.5 km (Taylor et al. 2010). However, other studies have documented stable flies dispersing much greater distances (Hogsette and Ruff 1985). Possibilities include large areas with low-quality developmental substrates and low densities of stable fly larvae such as rotting vegetation found in croplands and grasslands. Most efforts for locating and identifying stable fly developmental habitats have been directed at sites associated with livestock production where vegetative materials are contaminated with livestock wastes. These sites can have immature stable fly densities from 10 to 30 thousand larvae per m^2 (Patterson and Morgan 1986, Broce et al. 2005) but usually occupy limited areas < 500 m^2. Low-population density but high area sites may have been overlooked because finding sparsely dispersed developmental sites would require long searches beyond the scope of affected premises. Stable fly larvae have rarely been observed in such substrates in the United States, but have been observed developing in cropland residues in other countries such as Western Australia, Brazil, and Costa Rica (Cook et al. 2011, Dominghetti et al. 2015, Solorzano et al. 2015). Importantly, if a significant proportion of the stable flies are developing in habitats not associated with livestock, primary stable fly management recommendations (i.e., sanitation in livestock facilities) will not effectively reduce stable fly populations. Research continues to explore these possibilities.

Relative to the procedures outlined herein, the bucket emergence traps are easier to make, handle/transport, and install than the wood framed traps used previously. These traps are also more durable and do not rot after extended contact with decomposing vegetation or animal wastes. The collecting area of the bucket traps is somewhat smaller than that of the wood framed traps (530 vs 2,500 cm^2) meaning that more traps must be employed to sample an equivalent area.

Over the past several years, Alsynite has become more and more difficult to obtain. The current study continued to use Alsynite traps in order to maintain continuity in the long-term, 17 yr, trapping

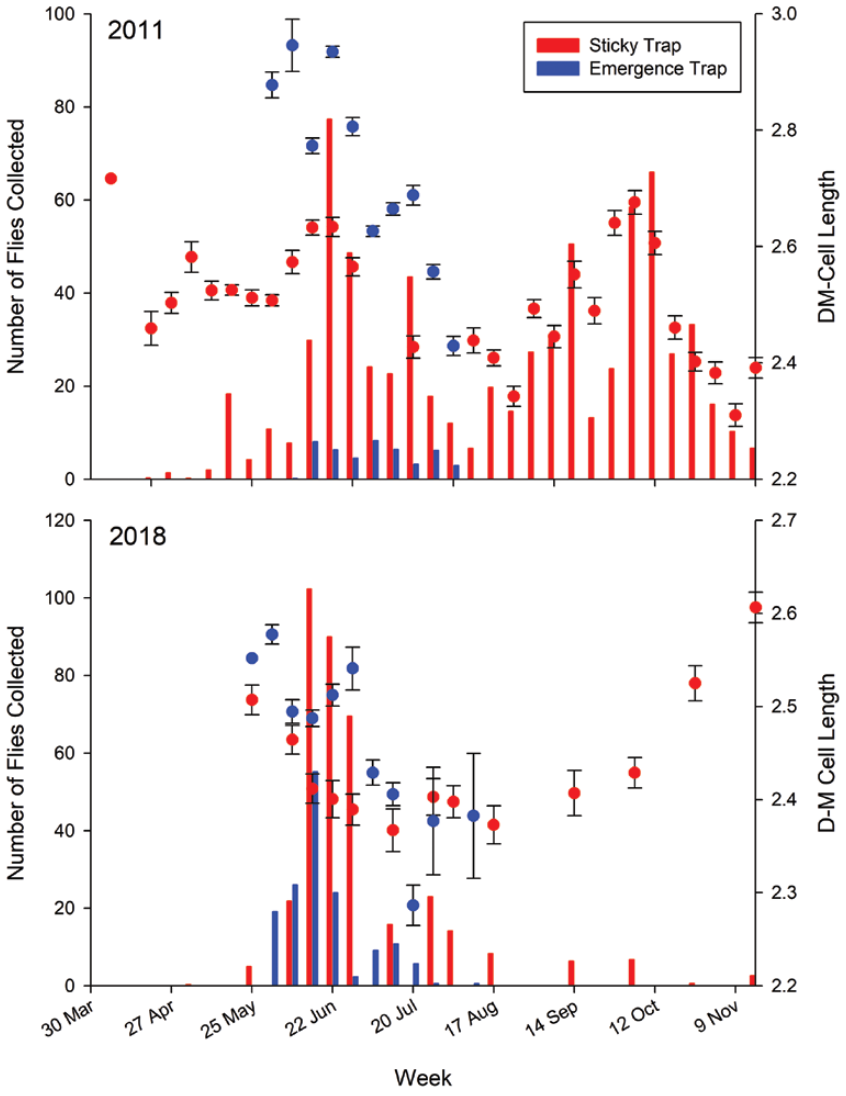

Fig. 5. Mean trap catches (bars) and wing sizes (circles) for stable flies collected emerging from winter hay feeding sites (blue) and collected on sticky traps (red) at the Eastern Nebraska Research and Extension Center, in 2011 and 2018. Sticky trap numbers are mean number of stable flies collected per day per trap. Emergence trap numbers are mean number of stable flies collected per trap per week. Wood frame emergence traps were used in 2011, and bucket emergence traps were used in 2018. Error bars for wing size are SEM.

studies conducted at ENREC. For new studies, Coroplast traps (Beresford and Sutcliffe 2006) are being substituted for Alsynite traps. Although Coroplast traps are more effective without sleeves, applying the adhesive directly to the Coroplast and disposing the traps after use, they can be used with sleeves to reduce costs (DBT, unpublished data) as well.

A disadvantage of using diluted Tangle-trap as an adhesive is that occasionally, especially during warm weather, some flies were observed escaping from the traps. Applying the Tangle-trap is messy and labor intensive as well. Other adhesives, such as preglued sleeves, provided by Olson (Olson Products Inc., Medina, OH) are more effective and convenient. However, it is not possible to remove intact flies from those adhesives for further processing. If the goal of trapping is for management, alternative adhesives may be more effective than the Tangle-trap.

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