Improved Sentinel Method for Surveillance and Collection of Filth Fly Parasitoids

Christopher J. Geden

Dana M. Johnson

David B. Taylor

OXFORD

Improved Sentinel Method for Surveillance and Collection of Filth Fly Parasitoids

Christopher J. Geden,[1,3,] **Dana M. Johnson,**[1] **and David B. Taylor**[2,]

[1]USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Dr, Gainesville, FL 32608, [2]USDA, ARS, Agroecosystem Management Research Unit, Lincoln, NE 68583-0937, and [3]Corresponding author, e-mail: chris.geden@ars.usda.gov

Disclaimer: Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. USDA is an equal opportunity provider and employer

Abstract

Parasitoids are important natural enemies of house flies and other muscoid flies. The two most commonly used methods for collecting fly parasitoids from the field have distinct advantages and disadvantages. Collections of wild puparia depend on the ability to find puparia in sufficient numbers and are prone to localized distortions in relative species abundance because of the overrepresentation of samples from hot spots of fly larval activity. Placement and retrieval of sentinel puparia is convenient and allows consistent sampling over time but is strongly biased in favor of *Muscidifurax* spp. over *Spalangia* spp. An improved sentinel method is described that combines some of the advantages of these two methods. Fly medium containing larvae is placed in containers, topped with a screen mesh bag of puparia, and placed in vertebrate-proof wire cages. Cages are placed at sites of actual or potential fly breeding and retrieved 3–7 d later. The modified method collected species profiles that more closely resembled those of collections of wild puparia than those from sentinel pupal bags. A method is also described for isolating puparia individually in 96-well tissue culture plates for parasitoid emergence. Use of the plate method provided a substantial saving of time and labor over the use of individual gelatin capsules for pupal isolation. Puparia from the collections that were housed individually in the wells of tissue culture plates had a higher proportion of emerged *Spalangia* species than puparia that were held in groups.

Key words: house fly, parasitoid, sampling, *Muscidifurax*, *Spalangia*

House flies (*Musca domestica* L. [Diptera: Muscidae]) and stable flies (*Stomoxys calcitrans* L. [Diptera: Muscidae]) are attacked by several species of pupal parasitoids, some of which are available as commercial products for release as part of IPM programs (reviewed in Machtinger et al. 2015a, Machtinger and Geden 2018). Field-collected parasitoids are sometimes needed either to start new colonies or as part of monitoring programs to determine parasitism rates and the relative abundance and preferences of the parasitoid species. Parasitoids can be collected/monitored by collection of wild puparia from active fly development sites on farms and holding them in the lab for fly and parasitoid emergence. Because the collection of wild puparia relies on the researcher being able to find fly puparia and the tendency of such sites to be transitory, it is difficult to resample sites over time. Sites with larval activity often appear and disappear between farm visits. Moreover, the patchy distribution of immature flies can skew relative species abundance data because of local overrepresentation.

An alternative collection method proposed by Rutz and Axtell (1980) uses 'sentinel bags', wherein 30–50 lab-reared live fly puparia

are added to screen mesh bags that are then placed in field sites with actual or potential fly larval development. Freeze-killed puparia have been used instead of live hosts in some studies (Floate et al. 1999, McKay and Galloway 1999, Gibson and Floate 2004), but developmental success of *Spalangia* (Hymenoptera: Pteromalidae) parasitoids on such hosts is much lower than in live puparia (Floate 2002, Geden and Kaufman 2007, Kaufman and Geden 2009). The primary advantage of the sentinel method is that it allows monitoring the same locations over time and does not rely on the availability of wild fly puparia. The sentinel pupal bag method is easy to use, inexpensive, and requires little on-farm time. Puparia can be processed and held for emergence in a short time because of the modest numbers involved, typically 300–500/farm/wk. Puparia that have been returned from the field using this method are held for adult parasitoid emergence either together in groups or placed individually in small cups or gelatin capsules. Isolation of individual puparia may prevent faster developing *Muscidifurax* spp. (Hymenoptera: Pteromalidae) from parasitizing puparia containing slower-developing *Spalangia*

spp. immatures before they can complete development. To our knowledge, there is no published information on whether isolating individual puparia has an effect on the relative proportions of *Spalangia* and *Muscidifurax* emerging from samples.

Both methods have advantages and disadvantages, as outlined in Table 1. Perhaps the most significant is that the two methods give different pictures of relative species abundance. Rutz and Axtell (1980), working in North Carolina poultry houses, first noted that sentinel bags collect proportionally more *Muscidifurax* and fewer *Spalangia* parasitoids than the collection of wild puparia. This divergence was confirmed in subsequent studies on Nebraska feedlots (Meyer and Petersen 1982, Petersen and Watson 1992) and California dairy farms (Meyer et al. 1990). *Muscidifurax* spp. forage near the surface of the substrate, whereas *Spalangia* spp. are better at finding buried puparia (Floate and Spooner 2002, Geden 2002). *Muscidifurax* spp. are therefore more likely to encounter sentinel bags, which are placed on or just below the surface to facilitate finding them on subsequent visits. Moreover, *Muscidifurax raptor* is attracted to odors emanating from house fly puparia, whereas *S. cameroni* is 'blind' to pupal odors but sensitive to odors associated with fly larvae (Machtinger et al. 2015b).

In this report, we describe a modified sentinel method that combines the convenience and repeatability of the sentinel bag method

Table 1. Advantages and disadvantages of current fly parasitoid sampling methods

	Sentinel pupal bags	Collection of wild puparia
Advantages	Can sample same locations over time	More realistic indicators of parasitoid activity
	Not dependent on fly populations at site	Detects species that attack larvae and young puparia
	Easy to deploy and process	Samples are always from actual (not potential) breeding sites
	Allow user to choose host species (house fly, stable fly, *Fannia* spp., *Hydrotaea* spp.)	
Disadvantages	Biased in favor of *Muscidifurax* spp.	May be biased in favor of *Spalangia* spp.
	Not always located near fly breeding sites and areas of parasitoid activity	Dependent on fly populations
	Small number of hosts	Sometimes difficult to find puparia
	Cannot detect species that attack larvae and young puparia	Unequal sample sizes, overrepresentation from hot spots
	Larval and infested habitat odors are not present	Difficult to sample same locations over time
		Laborious to collect and process

Table 2. Materials needed to assemble and process sentinel stations for filth fly parasitoids

	Item	Example source
Fly rearing	House fly colony	Various research laboratories, online from Carolina Biological (2700 York Road, Burlington, NC 27215-3398)
	Wheat bran	Local farm store or online (Example: F-R-M Feeds, Flint River Mills, Inc., 1100 Dothan Road, P. O. Box 280, Bainbridge, GA, 39818-0280)
	Calf Manna	Local farm store or online (Manna Pro Products, 707 Spirit 40 Park Drive #150, Chesterfield, MO 63005)
	Fly larval rearing tray (56 × 43.5 × 8 cm)	Del-Tec Packaging, 4020 Pelham Court, Greer, SC 2965
	King size pillowcases	Example: Walmart Mainstays 200 thread count percale, available in stores or online (www.Walmart.com)
Assembly of sentinel stations	Fiberglass standard window screening	Home or hardware store
	Food storage container with lid, 1–1.25 quart	Example: Ziploc medium square containers, widely available
	Cotton muslin squares (19 cm) to cover containers after collection	Local fabric stores or numerous online sources
	Teflon dispersion to paint or dip containers to exclude ants	Fluon Insect-a-Slip Insect Barrier, (www.Bioquip.com)
	Live animal trap with sufficient room for sentinel containers.	HAVAHART medium 2-door animal trap Model #: 1030-B, farm stores or online
	Concrete blocks or other solid heavy items to place on tops of cages (minimum coverage 61*19 cm) for wind and rain protection	Home, building or garden supply stores.
Holding puparia in the laboratory	96-well tissue culture plates	Example: Falcon Microtest U-Bottom plates, Corning 351177 (www.Fishersci.com)
	Heavy card stock paper	Office supply stores or online
	Parafilm, roll of 4 × 125 in.	Bemis PM996 (www.Fishersci.com)
	Masking tape to seal plates	Home, hardware, paint stores

with the more complete species spectrum representation of wild pupal collections. Data are also presented on the effect of holding conditions for puparia (in groups or individually) on the relative species composition of emerged adult parasitoids.

Experimental Design

The method consists of placing fly larval medium with fly larvae in plastic containers (approximately 1,000 larvae each), topping the media surface with a screen bag containing approximately 1,000 puparia, and placing them in the field. The sentinel containers are placed inside a wire small mammal trap to exclude disturbance by vertebrates. Cages are placed near areas of actual or potential fly development for 3–7 d and serve as mobile 'hot spots' of fly development. Containers are returned to the lab, puparia are held 5–10 d for fly emergence, and uneclosed puparia are isolated individually in 96-well tissue culture plates for parasitoid emergence. Materials and sources are presented in Table 2. The results presented below are from field studies at a dairy farm in Gilchrist County, FL, and a beef cattle research farm in Nebraska. In these tests, the modified sentinel method was compared with collections of wild puparia and the placement of conventional sentinel bags of 50 live house fly puparia. Wild puparia were collected from several locations at each farm per visit. Where possible, at least 200 puparia were collected per location/farm/visit.

Procedure

Preliminary steps include producing fly larvae and puparia and preparing the containers for the addition of media. Fly larval trays are prepared by combining 6,500 cm³ of wheat bran, 500 cm³ Calf Manna (Manna Pro Products, Chesterfield, MO), and 3.75 liters of tap water. Eggs are collected from colony cages, shaken in water to break up clumps, and transferred to conical tubes to a volume of 2 cm³ of settled eggs. This volume is equal to approximately 20,000 eggs. Eggs are transferred to the surface of the medium (2 cm³/tray), then the tray is covered with a pillowcase and placed in a rearing room maintained at 29°C. Pillowcases are used to mitigate drying of the medium, to prevent oviposition by loose flies in the rearing facility, and to deter entry by stray parasitoids. Puparia are removed from rearing medium by water flotation 6–7 d after egg placement, dried, and held at 12°C for up to 7 d before use as sentinels. Puparia should be harvested as soon after pupation as it is practical to account for the range in puparial ages that different species prefer to parasitize. Larvae are collected with their associated rearing medium 4–5 d after egg placement, after thoroughly mixing the medium and larvae to distribute the larvae as evenly as possible. Using this volume of medium and rate of egg loading, 750 cm³ contains approximately 1,000 larvae.

The plastic containers used to house the sentinel medium, larvae, and puparia are 1.25-quart (1,183 ml) food storage containers that are sold, with matching lids, in most grocery stores. The outer surface of the containers is coated with a Teflon dispersion to deter ants

Fig. 1. Untreated plastic container (left) and plastic container after coating with Teflon dispersion to exclude ants (A); container after the addition of fly larvae in rearing medium (B).
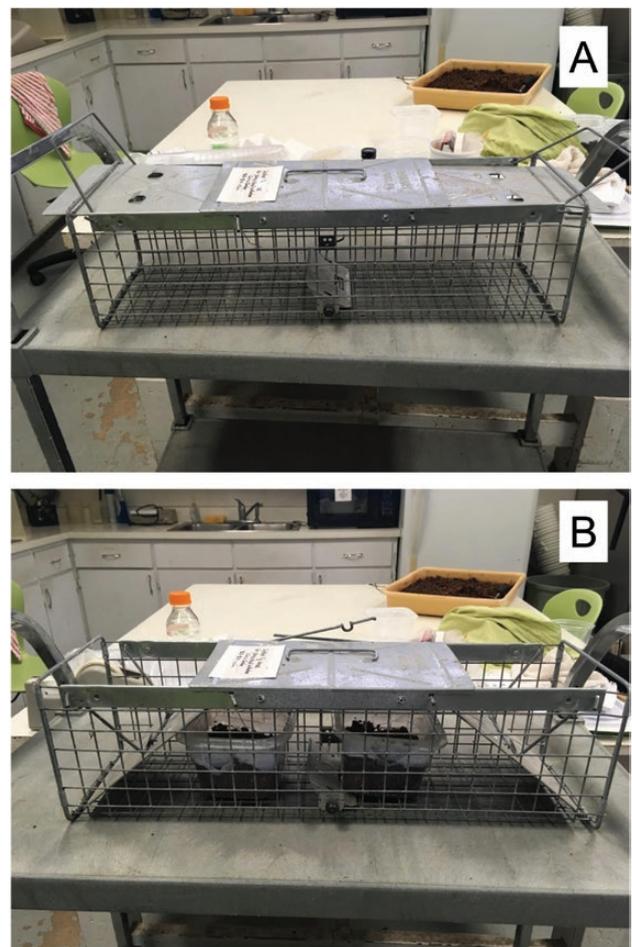

Fig. 2. Mammal trap before (A) and after (B) placement of containers of sentinel fly immatures and securing the trap doors to prevent animal entry.

from entering them (Fig. 1). Rearing medium, 750 cm³ containing approximately 1,000 third instars, is added to each container. Screen bags are filled with 30 cm³ of puparia (approximately 1,000), closed with a binder clip, and placed on the surface of the larval rearing medium. Containers are placed inside medium-size live mammal traps with the trap doors secured in the closed position to exclude mammal entry (Fig. 2). Traps with modified sentinels are placed in areas of actual or likely fly larval activity and can be covered to protect against wind and rain (Fig. 3).

Sentinel containers that have been in the field are easily swapped out with fresh containers with minimal disruption to the placement of

Fig. 3. Examples of collection sites with assembled sentinel stations.

the outer mammal trap. Containers are covered with cotton muslin at the time of collection and returned to the lab. Sentinel puparia are placed in cages for fly emergence. The entire volume of larval rearing medium in the plastic container is transferred to a large plastic bowl for water flotation. The puparia that have developed are collected, dried and held in cages for fly emergence. If wild puparia are collected as well, they need to be isolated individually (next section) as soon as possible because parasitoid emergence can begin at any time after collection. Wild puparia can also be stored at 12–14°C after collection to delay emergence until time is available to isolate them individually.

If the purpose of collection is to establish colonies of parasitoids, the unenclosed puparia can be pooled and held for parasitoid emergence at 27°C. The window for parasitoid emergence is broad, starting at 2 wk from placement for the first *Muscidifurax* males to as long as 6 wk for some *Spalangia* females. It is important to check puparia daily and remove adult parasitoids, as *Muscidifurax* spp. females can kill immature *Spalangia* by parasitizing puparia that contain developing parasitoids. Adults can be removed individually with an aspirator or by placing the puparia with parasitoids in a sieve and shaking them onto a chill table. A US standard #10 sieve is ideal as the 2 mm mesh opening is the largest of the standard sieve series that will retain house fly puparia. A #12 sieve is recommended for stable fly, *Hydrotaea* spp. (Diptera: Muscidae), and undersized house fly puparia. Small sieves can be fashioned by cutting disks of appropriately sized hardware cloth and inserting the disk into the lid of a paper can.

Chilled parasitoids can be identified and sorted into groups of the same species to start a colony. The key by Gibson (2000a) is an excellent resource for most species in North America. The illustrated key by Rueda and Axtell (1985) is also helpful but is long out of print. Additional keys are available for the genera *Urolepis* (Hymenoptera: Pteromalidae) (Gibson 2000b) and *Trichomolopsis* (Gibson and Floate 2001). The two most common genera, *Spalangia* and *Muscidifurax*, are easily separated. *Spalangia cameroni* Perkins and *S. endius* Walker have sufficiently distinct features that they are readily recognized, and *S. drosophilae* Ashmead are much smaller than any of the common species. Identifying *S. nigroaenea* Curtis and *S. nigra* Latreille is more difficult and takes time and practice. Similarly, distinguishing *M. raptor* Girault and Sanders from *M. zaraptor* Kogan and Legner is challenging. It is helpful to obtain identified reference specimens to examine the character states described in the published keys.

If the purpose of the collection is to monitor parasitism and relative species abundance, unenclosed puparia should be isolated in individual containers to avoid the above-mentioned issue of early-emerging *Muscidifurax* adults killing the immatures of slower-developing *Spalangia* spp. Isolation is also warranted if gregarious species such as *M. raptorellus* Kogan and Legner, *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae), or some *Trichomalopsis* (Hymenoptera: Pteromalidae) species are present. This is most commonly done using gelatin capsules. The standard #2 gelatin capsule (length 15.3 mm, volume 0.37 ml) is the smallest size capsule than can be easily opened and closed by hand and, therefore, the most space-efficient size for storing large numbers of capsules. The larger #00 (length 20.2, volume 0.91 ml) is useful for larger hosts such as *Sarcophaga bullata* (Parker) (Diptera: Sarcophagidae) and for individuals who find it difficult to manipulate the smaller capsules. Capsules can be purchased in small batches from health food stores. Larger quantities can be purchased from online suppliers but may require documentation describing their intended use.

Placing puparia in gelatin capsules ('gel-capping') can be prohibitively time-consuming if sample sizes are large. An experienced person working at full speed requires about 5 s per pupa, so it can take nearly 90 min to isolate 1,000 puparia. Most people find that fatigue of the
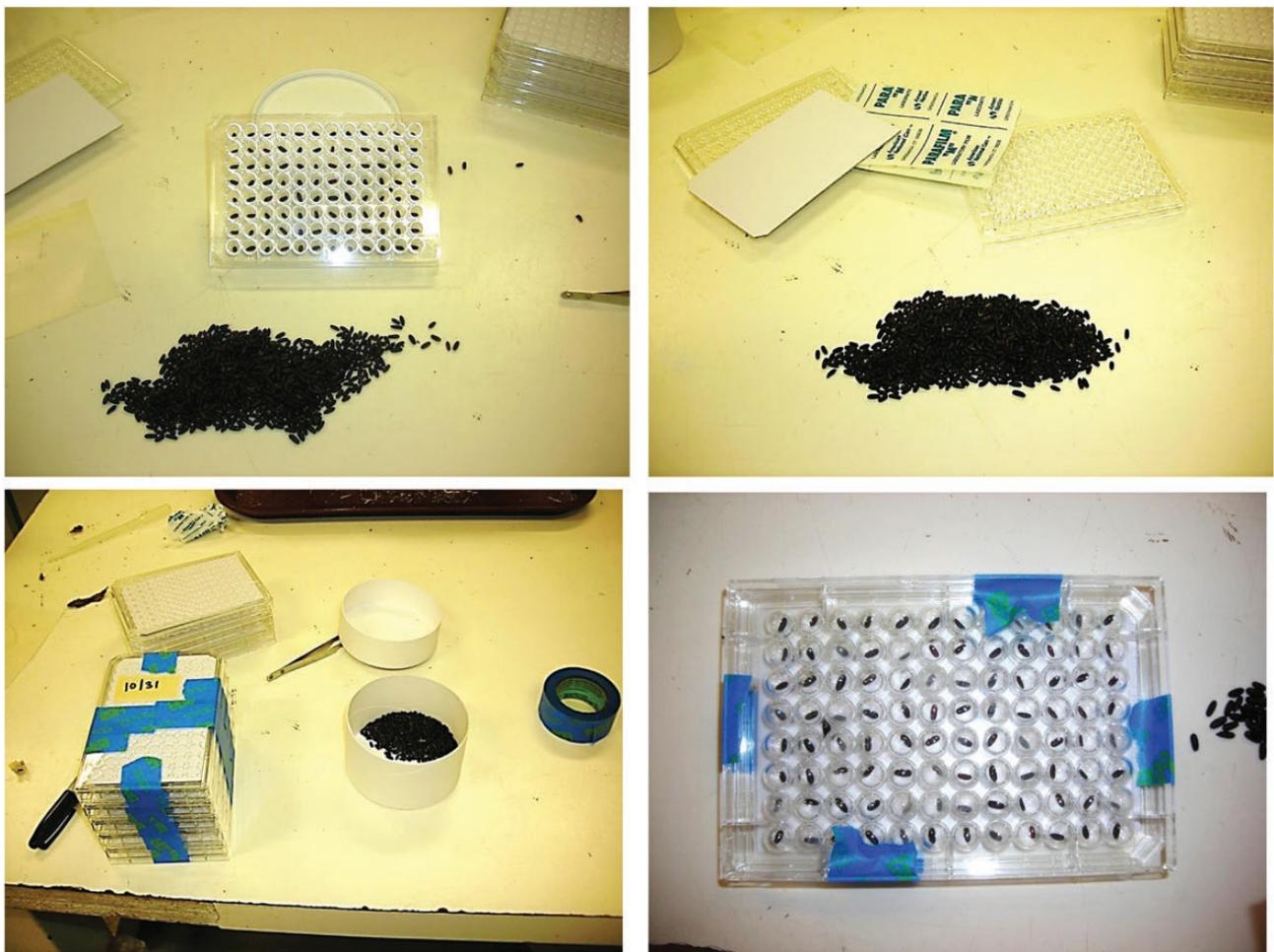
Fig. 4. 96-well tissue culture plates used to house individual fly puparia for parasitoid emergence.

finger muscles, along with boredom, result in much slower gel-capping rates. Moreover, the same laborious process must be done in reverse after parasitoid emergence to allow species identification.

Tissue cultures dishes with 96 wells provide an alternative to gel-capping. Puparia can be placed in the wells at a fraction of the time required to open and close gelatin capsules. However, the lids of the dishes are designed to allow gas exchange and the resulting gap between the lid and the top of the well is large enough for parasitoids to escape. To form a tight seal, a piece of Parafilm (Bemis Co., Neenah, WI) is cut and placed so that it fits flush over the tops of the wells (Fig. 4). Heavy cardstock paper is then cut and placed over the Parafilm. The card needs to be cut carefully so that it fits within the inner ridge of the lid, as this ridge is the cause of the gap. The lid of the dish is placed over the card, squeezed tightly, and secured with a piece of tape on each side (Fig. 4). A similar arrangement can be constructed using several layers of paper towels if Parafilm and cardstock are not available, but the seal will not be as tight.

The sealed plates are then held at 24–28°C for parasitoid emergence, which can be checked by examining the underside of the plates for adults. If no gregarious species are present (i.e., no well contains more than one adult parasitoid), the lids can be removed, the puparia and dead parasitoids tapped out, and the parasitoids separated from the puparia either by hand or the use of a sieve as described previously.

There are no published accounts that we are aware of documenting the necessity for isolating puparia. While doing the evaluations of the modified sentinel stations described here, we compared the apparent relative species abundance in Florida samples that had been isolated with those that had not. This was done by dividing puparia from single sentinel containers into groups that were either isolated using tissue culture plates or pooled and held for group parasitoid emergence. After eliminating samples with either very low parasitism or only a single genus of parasitoid, 30 samples were identified that included specimens of both *Muscidifurax* spp. and *Spalangia* spp. in the isolated group. The relative abundance of the species present in the isolated samples was compared with relative abundance in companion sub-samples where puparia were held in groups. A total of 7,157 adult parasitoids were included in these samples.

Results

Comparisons of the three sampling methods (wild pupal collections, sentinel pupal bag, and the modified sentinel system) are shown in Tables 3–4 and Tables 5–6 for field sites in Florida and Nebraska, respectively. Puparia from the sentinel pupal bag method had much lower parasitism than the other two methods at both locations. Perhaps the most striking difference in these tests was the percentage of collected parasitoids that were *Spalangia* spp. In the Florida samples, *Spalangia* spp. made up 16.9% of the parasitoids collected using sentinel pupal bags compared to 86.5% for the wild-collected puparia. Parasitoids from Florida sentinels in the modified method that were placed as larvae were 76.8% *Spalangia* spp, whereas only 15.9% of the pupal sentinels in the same containers were *Spalangia* spp. Sentinel puparia

Table 3. Parasitism of house fly puparia determined by different methods at a Florida dairy farm, May–June 2012

	Wild pupal collections	50 puparia in conventional sentinel bags	Modified sentinels placed as	
			Puparia	Larvae
No. puparia	1,152	4,500	90,000	90,000
% uneclosed puparia	76.4	20.0	10.6	32.8
% parasitized puparia	12.3	4.5	4.1	15.6
% samples parasitized	100	12.2	55.6	78.9

Table 4. Percent relative abundance of house fly parasitoids collected using different methods, Florida dairy, May–June 2012

Species	Wild pupal collections	50 puparia in conventional sentinel bags	Modified sentinels placed as	
			Puparia	Larvae
Muscidifurax raptor	13.1	20.2	53.1	17.7
Pachycrepoideus vindemmiae	0.5	62.8	30.9	5.5
Spalangia cameroni	37.8	2.7	6.6	20.1
S. endius	28.6	14.2	7.7	46.1
S. nigroaenea	19.6	0.0	0.2	6.7
S. nigra	0.5	0.0	0.4	2.3
S. drosophilae	0.0	0.0	1.0	1.6
% total *Spalangia*	86.5	16.9	15.9	76.8
Total no. parasitoids	444	549	3,690	14,040

Table 5. Parasitism of house fly puparia determined by different methods at a Nebraska cattle facility, June 2012

	Wild pupal collections	50 puparia in conventional sentinel bags	Modified sentinels placed as	
			Puparia	Larvae
No. puparia	4,486	2,000	40,000	40,000
% uneclosed puparia	87.2	36.2	14.6	22.4
% parasitized puparia	15.2	5.5	4.1	4.1
% samples parasitized	100.0	32.5	62.5	65.0

Table 6. Percent relative abundance of house fly parasitoids collected using different methods, Nebraska cattle facility, June 2012

Species	Wild pupal collections	50 puparia in conventional sentinel bags	Modified sentinels placed as	
			Puparia	Larvae
Muscidifurax raptor	25.4	17.3	17.0	23.4
M. zaraptor	15.4	78.2	77.2	43.6
Spalangia cameroni	4.8	0.0	1.0	2.6
S. endius	5.6	0.0	1.2	6.9
S. nigroaenea	11.2	0.0	0.3	6.6
S. nigra	37.5	4.5	1.8	14.1
S. drosophilae	0.0	0.0	0.3	1.8
Urolepis rufipes	0.0	0.0	1.2	0.0
Trichomolpsis spp.	0.0	0.0	0.0	1.1
% total *Spalangia*	59.1	4.5	4.6	32.0
Total no. parasitoids	682	110	1,640	1,637

showed much higher percentages of *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) than either wild puparia or sentinels that were placed as larvae.

The species composition of the Nebraska collections differed somewhat from the Florida samples, but the overall trend was similar. The highest percentage of *Spalangia* spp. was observed in the wild pupal collections (59.1%) and in sentinels placed as larvae (32%). Fewer than 5% of sentinels placed as puparia were parasitized by *Spalangia* spp., and the majority of parasitoids emerging from sentinel puparia were either *M. raptor* or *M. zaraptor*.

Modified sentinels were more sensitive than the other methods to some relatively uncommon species. *Spalangia drosophilae*, *Urolepis rufipes* (Ashmead), and *Trichomalopsis* spp. were only collected using the modified sentinel method.

Comparison of parasitoid emergence from samples where puparia were isolated individually or held in groups is presented in Table 7. *Spalangia* spp. (mostly *S. cameroni* and *S. endius*) made up nearly half of the emerged parasitoids from isolated puparia, but only 28.7% of the parasitoids that emerged from puparia held in groups. The proportion of *Spalangia cameroni* was nearly three times higher when puparia were isolated individually (14.9%) than when puparia were not isolated (5.5%).

Discussion

Results presented here confirm the observations of other studies that the proportion of *Spalangia* spp. collected using traditional sentinel pupal bags is much lower than in collections of wild puparia (Rutz and Axtell 1980, Meyer and Petersen 1982, Meyer et al. 1990, Petersen and Watson 1992). Differences in searching behavior and olfactory cues used for host location presumably account for some of this difference (Geden 2002, Machtinger et al. 2015b). Variation in development time may play a role as well. Wild puparia

Table 7. Relative abundance (% of total adults emerged) of parasitoids from puparia that were held either individually isolated or in groups

Species	Isolated puparia	Grouped puparia
Muscidifurax raptor	45.9 (5.8)	58.4 (5.4)
Pachycrepoideus vindemmiae	7.1 (2.6)	12.9 (3.7)
Spalangia cameroni	14.9 (3.4)	5.5 (2.7)
S. endius	27.4 (5.6)	20.7 (4.6)
S. nigroaenea	4.1 (1.5)	1.7 (0.8)
S. drosophilae	0.6 (0.4)	0.6 (0.4)
All *Spalangia* spp.	47.0 (7.5)	28.7 (5.8)

with developing parasitoids may have been parasitized on the day of collection or many weeks earlier. The longer development time of *Spalangia* spp. compared to *Muscidifurax* spp. would, therefore, be expected to favor higher proportions of *Spalangia* spp. in wild collections. It has been suggested that this leads to a bias in favor of *Spalangia* spp. with collections of wild puparia (Petersen 1986, Petersen and Watson 1992).

The modified sentinel method presented here bridges some of the differences between the traditional sentinel pupal bags and the collection of wild puparia in several ways that mitigate their respective biases. First, the fixed and relatively short exposure time in the field prevents long-term accumulations of slow-developing species. Second, the inclusion of larvae and their growth medium provides an opportunity to attract species such as *Spalangia cameroni* that focus on those cues. Third, the method uses 40 times more hosts than are typically used in sentinel bags, providing a more concentrated

source of host kairomones that may attract parasitoids from a wider distance.

Results presented in Tables 4 and 6 indicate that sentinels that are placed as larvae in larval medium produce adult parasitoids in proportions that are comparable to what emerges from collections of wild puparia. We were surprised that the proportion of *Spalangia* spp. emerging from 1,000 sentinel puparia placed on the surface of that medium was nearly identical to samples of traditional sentinel pupal bags containing 50 clean puparia with no larval cues. This observation supports the idea that host location by *Spalangia* spp. is driven by a combination of olfactory cues emanating from larvae and a propensity to search below the surface of the substrate. The results also suggest that the placement of sentinel puparia on the surface of the larval medium could be omitted with little reduction of resolution of the species present, but additional testing is needed to confirm this.

The isolation of individual puparia after fly eclosion is a time-consuming step. Nonetheless, results in Table 7 demonstrate that failure to isolate puparia distorts apparent species proportions. Not isolating puparia favors *Muscidifurax* spp. because of their shorter development time and aggressive parasitizing behavior. Floate et al. (1999) described the use of 96-well ELISA plates for isolating puparia, and the method has been used by several others (McKay and Galloway 1999, Noronha et al. 2007). As stated previously, we found that the gap between the lid of the plate and the top of the individual wells allowed some parasitoids to escape from their respective wells. We recommend the additional steps of adding parafilm and cardstock to form a parasitoid-proof gasket and fastening the lids with tape for a tight seal. The use of tissue culture plates for this purpose represents a major time-saving advantage over the use of gelatin capsules. The plates offer additional advantages of being stackable and easy to label and curate pending identification of specimens.

In summary, the modified sentinel method described here presents a significant advantage over traditional sentinel pupal bags by providing a more real realistic and complete profile of the parasitoid species present. Large numbers of hosts are required to place and process the sentinel units, but house flies are easy and inexpensive to produce. The quantities needed do not impose a significant burden on research programs that maintain robust fly colonies for other purposes. Moreover, the large number of hosts results in much higher yields of adult parasitoids for monitoring or initiating new colonies.

The modified sentinel method also has advantages over collections of wild puparia, which are subject to constant changes in availability of puparia to collect. The presence of media and larvae means that the units are essentially mobile fly developmental 'hot spots' that can be placed anywhere with actual or potential populations of fly immatures. Further work is needed to determine the utility of this method in indoor situations such as poultry houses and whether pupal sentinels on the medium surface can be eliminated without sacrificing the utility of the information gained.

Acknowledgments

References Cited

Floate, K. D. 2002. Production of filth fly parasitoids (Hymenoptera: Pteromalidae) on fresh and on freeze-killed and stored house fly pupae. Biocontrol Sci. Technol. 12: 595–603.

Floate, K. D., and R. W. Spooner. 2002. Parasitization by pteromalid wasps (Hymenoptera) of freeze-killed house fly (Diptera: Muscidae) puparia at varying depths in media. J. Econ. Entomol. 95: 908–911.

Floate, K., B. Khan, and G. Gibson. 1999. Hymenopterous parasitoids of filth fly (Diptera: Muscidae) pupae in cattle feedlots. Canadian Entomol. 131: 347–362.

Geden, C. J. 2002. Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies, *Musca domestica* L. (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31: 411–417.

Geden, C. J., and P. E. Kaufman. 2007. Development of *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) on live house fly (Diptera: Muscidae) pupae and pupae killed by heat shock, irradiation, and cold. Environ. Entomol. 36: 34–39.

Gibson, G. A. P. 2000a. Illustrated key to the native and introduced chalcidoid parasitoids of filth flies in America north of Mexico (Hymenoptera: Chalcidoidea). http://canacoll.org/Hymenoptera/Staff/Gibson/PDFs/chalkey.pdf (accessed 30 January 2020).

Gibson, G. A., 2000b. Differentiation of the species of *Urolepis* (Hymenoptera: Chalcidoidea: Pteromalidae), potential biocontrol agents of filth flies (Diptera: Muscidae). Canadian Entomol. 132: 391–410.

Gibson, G. A. P., and K. Floate. 2001. Species of *Trichomalopsis* (Hymenoptera: Pteromalidae) associated with filth flies (Diptera: Muscidae) in North America. Canadian Entomol. 133: 49–85.

Gibson, G. A. P., and K. D. Floate. 2004. Filth fly parasitoids on dairy farms in Ontario and Quebec, Canada. Canadian Entomol.136: 407–417.

Kaufman, P. E., and C. J. Geden. 2009. Development of *Spalangia cameroni* and *Muscidifurax raptor* (Hymenopter: Pteromalidae) on live and freeze-killed house fly (Diptera: Muscidae) pupae. Florida Entomol. 92: 492–496.

Machtinger, E. T., and C. J. Geden. 2018. Biological control of livestock pests: parasitoids. *In* C. Garros, J. Bouyer, W. Takken, R. C. Smallegange (eds), Ecology and control of vector-borne diseases, volume 5. Prevention and control of pests and vector-borne diseases in the livestock industry. Wageningen Academic Publishers, Wageningen, the Netherlands.

Machtinger, E. T., C. J. Geden, P. E. Kaufman, and A. M. House. 2015b. Use of pupal parasitoids as biological control agents of filth flies on equine facilities. J. Integr. Pest Manag. 6: 16. doi:10.1093/jipm/pmv015.

Machtinger, E. T., C. J. Geden, P. E. Teal, and N. C. Leppla. 2015a. Comparison of host-seeking behavior of the filth fly pupal parasitoids, *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Environ. Entomol. 44: 330–337.

McKay, T., and T. D. Galloway. 1999. Survey and release of parasitoids (Hymenoptera) attacking house and stable flies (Diptera: Muscidae) in dairy operations. Canadian Entomol. 131: 743–756.

Meyer, J. A., and J. J. Petersen. 1982. Sampling house fly and stable fly pupal parasites on beef feedlots and dairies in eastern Nebraska. Southwest. Entomol. 7: 119–124.

Meyer, J. A., B. A. Mullens, T. L. Cyr, and C. Stokes. 1990. Commercial and naturally occurring fly parasitoids (Hymenoptera: Pteromalidae) as biological control agents of stable flies and house flies (Diptera: Muscidae) on California dairies. J. Econ. Entomol. 83: 799–806.

Noronha, C., G. A. P. Gibson, and K. D. Floate. 2007. Hymenopterous parasitoids of house fly and stable fly puparia in Prince Edward Island and New Brunswick, Canada. Canadian Entomol. 139: 748–750.

Petersen, J. J. 1986. Evaluating the impact of pteromalid parasites on filth fly populations associated with confined livestock installations. Misc. Publ. Entomol. Soc. Amer. 61: 52–56.

Petersen, J. J., and D. W. Watson. 1992. Comparison of sentinel and naturally occurring fly puparia to measure field parasitism by pteromalid parasitoids (Hymenoptera). Biol. Control 2: 244–248.

Rueda, L. M., and R. C. Axtell. 1985. Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure. NC Agric. Res. Serv. Tech. Bull. 278: 1–88.

Rutz, D. A., and R. C. Axtell. 1980. House fly parasites (Hymenoptera: Pteromalidae) associated with poultry manure in North Carolina. Environ. Entomol. 9: 175–180.