

# Impact of Exposure Length and Pupal Source on *Muscidifurax raptorellus* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) Parasitism in a New York Poultry Facility

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**ABSTRACT** Commercially obtained *Nasonia vitripennis* Walker and *Muscidifurax raptorellus* Kogan & Legner were released weekly for 12 wk into a high-rise, caged-layer poultry house. After the release period, parasitoids were sampled using sentinel house fly (*Musca domestica* L.) pupae that were either laboratory-reared or field-collected as larvae and exposed for 2, 4, 7, and 14 d. Parasitoid-induced mortality was observed in 31% of laboratory colony pupae and in 26% of field-collected pupae, whereas successful parasitism rates of 48 and 51% were observed from these pupal sources, respectively. Parasitism was primarily by *M. raptorellus* (88%), and *Muscidifurax raptor* Girault & Sanders (11%), while *N. vitripennis* accounted for <1%. Percent female progeny ranged from 43% in *M. raptorellus* to 76% in *N. vitripennis*. Parasitoid emergence from 2-d exposed sentinel pupae was the lowest of all treatments. Parasitoid emergence from 7-d exposed sentinel pupae was the highest of all treatments. We found no differences between pupal source, suggesting that when sampling for *M. raptor*, *M. raptorellus*, and *N. vitripennis*, in poultry facilities, pupal source is not a confounding factor.

**KEY WORDS** *Muscidifurax raptorellus*, *Nasonia vitripennis*, poultry, parasitoid, filth fly, biological control

PARASITOIDS (HYMENOPTERA: PTEROMALIDAE) of house flies, *Musca domestica* L., are a crucial component of a successful integrated fly management programs in poultry facilities (Morgan et al. 1975, Rutz and Axtell 1981). Many poultry producers augment their biological control program by incorporating weekly releases of commercially produced parasitoids after manure removal. Production of progeny after these augmentative parasitoid releases results in parasitoid "recycling." This benefits producers by increasing parasitoid presence in the house without the cost of additional parasitoid releases.

*Nasonia vitripennis* Walker, a parasitoid commonly found in New York poultry houses (Rutz and Scoles 1989, Henderson and Rutz 1991), is commercially available for house fly control. The advantages that *N. vitripennis* has over other parasitoids include that it is gregarious, producing from 4 to 8 adult parasitoids from each parasitized house fly pupa and it is inexpensive to rear commercially (Fried et al. 1990).

In recent years, research has focused on another parasitoid species, *Muscidifurax raptorellus* Kogan & Legner (Petersen and Cawthra 1995, Petersen and Currey 1996a, Tobin and Pitts 1999, Kaufman et al. 2001). This species originally was imported from Chile and has since been isolated from a beef cattle feedlot in Nebraska (Antolin et al. 1996). The gregarious nature of this parasitoid suggests excellent potential for

biological control of flies (Petersen and Currey 1996a). Although the gregarious nature of parasitoids has been reported as not being a desirable trait, with respect to such species as *Trichomalopsis* spp. (Dobesh et al. 1994). The gregarious nature of *M. raptorellus* is considered a very desirable trait because each female not only attacks a large number of fly pupae but each parasitized fly pupa also generally produces multiple offspring (Petersen and Currey 1996b), all of which contribute to parasitoid recycling, an important component to fly biological control. Commercial insectaries are now mass-rearing *M. raptorellus* for release in livestock and poultry facilities.

Kaufman et al. (2001) reported on the parasitism rates and effectiveness of *N. vitripennis* and *M. raptorellus* following individual and paired releases. In the absence of indigenous parasitoids *N. vitripennis* was found to be the more effective parasitoid in New York poultry houses. Most parasitism in the *M. raptorellus* release house was attributed to *N. vitripennis*, indicating that despite large weekly releases of *M. raptorellus*, this species was either unable to compete with established *N. vitripennis* populations or unable to establish at the given release rate.

Parasitoid impact on house fly populations can be sampled in agricultural settings in several ways. The means for sampling parasitoids has created some controversy. Each sampling method influences the species composition of the parasitoids that emerge (Legner 1983, Rutz 1986, Petersen and Watson 1992, Jones

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and Wienzierl 1997). The use of sentinel pupae involves live (Rutz and Axtell 1980) and dead (Floate et al. 1999) laboratory-strain house fly pupae held in screened bags and placed in the field. This method assures that pupae are of a known age and that parasitism occurred within a given time period. It is possible that because these pupae did not develop in the environment they did not acquire the host-seeking cues that indigenous parasitoids use in searching (Wylie 1958). This could ultimately select for commercially produced parasitoids.

A second method of sampling makes use of field-collected pupae (Rutz and Axtell 1980, Meyer et al. 1991, Petersen and Watson 1992, Jones and Weinzierl 1997). Naturally occurring house fly pupae are collected on farms, returned to the laboratory and held for parasitoid emergence. Although this provides for natural parasitism, the ages of the pupae are not known which could result in a sampling bias for parasitoid species with longer development times. Furthermore, sufficient numbers of pupae are often difficult to recover.

A third method utilizes pupal traps, where screen cylinders are filled with moist wood shavings. These traps provide a suitable pupation site for naturally occurring last instar house fly larvae that subsequently are subject to parasitism (Hogsette and Butler 1981). Recently, Tobin and Pitts (1999) developed a sampling method that combined the benefits of naturally occurring flies with the regulated age, quality and quantity aspects of laboratory-reared pupae. The authors collected late instar house fly larvae and allowed the larvae to pupate in the lab before returning the pupae to the manure pack.

Several studies have compared these various methods of sampling parasitoid activity. Merchant et al. (1985) evaluated three methods in poultry facilities and suggested that each method may lead to biased estimates of house fly pupae viability and parasitism. They further stated that the estimates obtained from pupal traps may be the least biased. Petersen and Watson (1992) compared sentinel and naturally occurring fly pupae in cattle feedlots. They suggested that the naturally occurring pupal method appeared to be more reliable and sensitive to the entire parasitoid complex, but that adequate numbers of samples were difficult to obtain. Petersen and Cawthra (1995) and Tobin and Pitts (1999) suggest that for first generation released *M. raptorellus*, laboratory-reared sentinel hosts were more suitable indicators of dispersal than field-collected conspecifics.

Typically, sentinel pupae are exposed for 7 d (Rutz and Axtell 1980, Petersen and Watson 1992, Floate et al. 1999, Tobin and Pitts 1999, Kaufman et al. 2001). This time period accommodates both the pupa to adult fly developmental period and weekly trips to the field. To date, no one has examined the impact on parasitism of shorter and longer pupal exposures.

Our objective was to determine the effect on attack and successful parasitism levels of two sources of sentinel house fly pupae exposed for 2, 4, 7, and 14 d in a high-rise, caged-layer poultry facility during a 5 wk

period following 12 weekly releases of *M. raptorellus* and *N. vitripennis*.

## Materials and Methods

*Muscidifurax raptorellus* were purchased from a commercial insectary integrated pest management (IPM Laboratories, Locke, NY), and a colony *N. vitripennis* was originally purchased from a commercial insectary (Arbico, Tuscon, AZ) and subsequently reared at Cornell University. Parasitized house fly pupae were placed into the poultry facility in 12 weekly releases between 5 May and 26 July 1999. The targeted parasitoid release rate was 13 *M. raptorellus* and 2.5 *N. vitripennis*/bird. Cornell University recommendations suggest that producers release four parasitoids/bird (Kaufman et al. 2000). Releases were conducted in a 3,000 bird conventionally ventilated high-rise, caged-layer poultry facility (12 by 28 m) on the Cornell University poultry research farm near Ithaca, NY. In this house, air is forced outside from the manure pit by pit-level exhaust fans and manure falls directly to the pit floor, resulting in wide, somewhat flat manure mounds. All manure was removed from the house in late March and again in late April 1999.

The number of parasitized pupae required to provide the desired number of parasitoids designated for each field release was estimated for each species from the number of parasitoids successfully emerging from five subsamples of 20 parasitized pupae. This was initially determined from a commercial preshipment 4 wk before the first field release. To determine the actual parasitoid emergence from each field release shipment (number of parasitoids released into each house), five subsamples, containing 20 pupae each, were removed from each weekly shipment and held in the laboratory for parasitoid emergence.

Parasitized pupae for field release were divided equally into two mesh bags (100 by 150 cm, mesh density 5.5 squares/cm) that were placed equidistantly and suspended 1.5 m above the floor from center support beams, throughout the manure pit. Bags were replaced 2 wk later by the current week's parasitized pupae shipment. Following removal of the release bags, two subsamples of 40 pupae were removed from each bag to estimate the number of pupae that successfully produced parasitoids and the number of parasitoids that had emerged. Pupae removed from release bags were examined for parasitoid emergence holes and based on the mean number of parasitoids recovered from pupae held in the laboratory (see above), the numbers of parasitoids that had emerged were estimated.

Successful parasitism rates were monitored weekly using the sentinel pupal bag method of Rutz and Axtell (1980). Sentinel bags (8 by 8 cm, mesh density 5.5 squares/cm), each containing 30 live (<2 d postpupariation) house fly pupae, were placed weekly on the surface, near the base of the manure pile. House fly pupae were obtained from two sources; the Cornell University laboratory colony (LAB) and a Cornell University poultry research farm field-collected

source (FC). The LAB strain had been reared for 18 yr under laboratory conditions (26°C, 45–55% RH, and a photoperiod of 16:8 (L:D) h. LAB larvae were reared on a diet of a 2:3:15:8 ratio of calf protein supplement, wood chips, bran and tap water, respectively, and adults were maintained on water, nonfat dry milk and sucrose. The FC pupae were obtained using a method similar to that of Tobin and Pitts (1999), where poultry manure infested with third instar house fly larvae was placed into a 5-liter plastic container on a Friday afternoon. The container was returned to the laboratory where dry wheat bran was placed over the manure and the container covered with an organdy screen and held under conditions described previously. On the following Monday, pupae were removed from the bran using sieves. This procedure resulted in pupae of approximately similar ages from each source. To ensure that pupae in the field-collected group were not parasitized, subsamples were held in the laboratory.

Beginning on 2 August and continuing for 5 wk, 10 groups of sentinel pupae (four bags containing LAB and four bags containing FC pupae per group) were placed flat on the surface near the base of the manure pile at random locations throughout the house. The position of collection day and source were randomized weekly. Pupal source was paired with collection day to create a 2 × 4 layout at each site. An additional four bags from each pupal source were held in an organdy screened 1.9-liter container to exclude parasitoids and served as field controls. One bag from each of the 10 locations and a field control bag were collected 2, 4, 7, and 14 d after placement.

The unclosed pupae from each bag were held individually in size 00 gelatin capsules (Pyramid, Prescott, AZ) in the laboratory for 8 wk to allow for both fly and parasitoid emergence. The total number of unclosed pupae, of successfully parasitized pupae, and of emerged parasitoids were recorded. Parasitoids were sexed and identified to species. Percentage of unclosed pupae and percent of pupae producing a parasitoid were calculated. The percentage of fly pupae successfully parasitized was calculated by dividing the number of pupae with emergence holes by the total number of pupae retrieved. Parasitoid-induced mortality was characterized as mortality attributed to parasitoid stinging of the host but where progeny were not produced (Petersen and Cawthra 1995). Percent parasitoid-induced mortality was determined by subtracting percent successful parasitism from the corrected percent unclosed pupae. Successful parasitism and unclosed pupae were corrected for control mortality (Abbott 1925) and percentages were arcsine transformed for statistical analysis. Data from the 10 sentinel bags for each treatment and exposure day were pooled. Percentage unclosed pupae, successfully parasitized, and parasitoid-induced mortality variables from the overall and the individual species data were analyzed using a multi-factorial analysis of variance (ANOVA) model (PROC GLM; SAS Institute 1996) to detect differences between the fixed effects of pupa source and time of exposure. The

interaction term pupa source \* exposure day was included in the model. Time of exposure data were tested for treatment differences (lsmeans/slice, SAS Institute 1996) with a Tukey's mean separation. Arithmetic means are presented in all tables.

## Results and Discussion

We used a high release rate of *M. raptorellus* based on the results obtained in an earlier study conducted in poultry manure pits (Kaufman et al. 2001), where release of *M. raptorellus* at four parasitoids per bird resulted in parasitism levels of only 7% or less. Therefore, we increased the *M. raptorellus* release level approximately threefold to 13 parasitoids/bird while maintaining *N. vitripennis* release levels at 2.5 parasitoids/bird (Kaufman et al. 2001) to determine if *M. raptorellus* would become the dominant species if provided an initial numerical edge. In data not presented, only 2% successful parasitism was observed from sampling conducted with LAB pupae during the first 5 wk of the release period, with no parasitism recorded during the first week. These limited observations support those of Tobin and Pitts (1999), who reported limited *M. raptorellus* movement from release sites when used in poultry facilities. However, in studies conducted in cattle feedlot pens *M. raptorellus* was recovered from sentinel pupae at distances as great as 100 m from the release site (Petersen and Cawthra 1995, Lysyk 1996, Floate et al. 2000). It should be noted that an enclosed poultry facility is quite different from an open cattle feedlot and our study was conducted in an environment very similar to that of Tobin and Pitts (1999). Therefore, these contradictory reports suggest that *M. raptorellus* searching behavior and resultant dispersal may be profoundly impacted by the macro- and microhabitats into which it is released. Further studies are needed to more closely examine the impact of habitat on *M. raptorellus* searching behavior.

During the 5 wk study, a total of 12,060 sentinel pupae, both LAB and FC, was placed in the poultry facility (Table 1). Parasitoid-induced mortality was recorded in 31% of LAB pupae and in 26% of FC pupae, whereas successful parasitism rates of 48 and 51% were observed from these pupal sources, respectively. A total of 35,335 parasitoids was recovered from sentinel pupae. Parasitism was primarily by *M. raptorellus* (88%), and *Muscidifurax raptor* Girault and Sanders (11%), whereas *N. vitripennis* accounted for <1%. Successful parasitism resulted in a mean of 6.6 and 6.3 *M. raptorellus* and a mean of 4.0 and 5.4 *N. vitripennis* progeny produced per pupa from LAB and FC pupae, respectively.

More than 77% of the sentinel pupae were killed and >50% of these pupae were successfully parasitized (Table 1). This high level of postrelease parasitoid activity suggests that parasitoid recycling was occurring. When released at a high rate for 12 wk, *M. raptorellus* became the dominant parasitoid recovered from sentinel pupae during the postrelease period, accounting for >97% of all parasitoids and >88% of all parasitized pupae (Table 1). Further investigations

**Table 1. Successful parasitism and mortality of laboratory-reared and field-collected sentinel pupae placed in a high-rise, caged-layer poultry facility following 12 wk of *Nasonia vitripennis* and *Muscidifurax raptorellus* releases**

	No. (% of total) collected	
	Laboratory colony	Field-collected
Pupae placed	6101	5959
Unclosed pupae	4879 (80.0)	4626 (77.6)
Parasitoid-induced mortality	1916 (31.4)	1544 (25.9)
Parasitized pupae	2963 (48.6)	3082 (51.7)
<i>Nasonia vitripennis</i> collected	111 (0.6)	130 (0.7)
Successfully parasitized pupae	30 (1.0)	24 (0.8)
<i>Muscidifurax raptorellus</i> collected	17,485 (98.1)	17015 (97.2)
Successfully parasitized pupae	2642 (89.2)	2696 (87.5)
<i>M. raptor</i> collected	294 (1.6)	369 (2.1)
Successfully parasitized pupae	294 (9.9)	369 (12.0)

*M. raptorellus* released weekly for 12 wk at 13 parasitoids per bird and *N. vitripennis* released at 2.5 parasitoids per bird prior to sentinel bag placement.

into a compromise level of release are needed to refine the use of this species in fly management systems. Based on these results, it is apparent that *N. vitripennis* was unable to establish in this poultry facility. Kaufman et al. (2001) documented high levels of *N. vitripennis* activity in poultry facilities that had received smaller numbers of *M. raptorellus*. Wylie (1972) reported that if parasitoid oviposition occurred before attack by a conspecific, the senior *N. vitripennis* or *Muscidifurax zaraptor* Kogan and Legner survived. Although *M. raptor* was not released and the manure had been removed from the facility before the start of the parasitoid releases, this native parasitoid accounted for 11% of the total successful parasitism.

Wylie (1976) suggested that the adult progeny of arrhenotokous parthenogenic Hymenoptera are typically 65% female. Percent female values in the current study varied with females making up only 43% in *M. raptorellus*, whereas 76% of recovered *N. vitripennis* were female. Wylie (1973) reported that *N. vitripennis* progeny were >70% female when the parental cohort was presented with unparasitized hosts; however, parasitoids laid a smaller percentage of female eggs on previously parasitized house fly pupae. As was observed in our study, Petersen and Currey (1996) also reported that *M. raptorellus* progeny production was male biased, with only 43% female progeny produced. Studies examining the impact of prior parasitism have not been reported for *M. raptorellus*.

Significant differences in overall successful parasitism were observed within the main effects model ( $F = 4.90$ ,  $df = 7$ ,  $P \leq 0.0008$ ). Differences were not observed between the pupal source, but, parasitoid emergence was lowest from 2-d exposed sentinel pupae ( $F = 10.17$ ,  $df = 3$ ,  $P \leq 0.0001$ ) (Table 2). A significant parasitoid-induced mortality effect was not observed in the main effects model ( $F = 0.79$ ,  $df = 7$ ,  $P \leq 0.6047$ ). Significant differences were observed in the main effects model for unclosed pupae ( $F = 4.30$ ,  $df = 7$ ,  $P \leq 0.0019$ ) (Table 2), but not between the pupal source. Fly emergence, as would be expected,

**Table 2. Successful parasitism, parasitoid-induced mortality, and killed sentinel house fly pupae exposed for various time periods in a New York high-rise, caged-layer poultry facility following release of *N. vitripennis* and *M. raptorellus***

Days exposed	% (SE)		
	Successful parasitism	Parasitoid-induced mortality	Unclosed
2	38.4 (3.33)b	31.4 (2.91)a	67.0 (2.87)b
4	52.7 (3.44)a	30.5 (2.35)a	80.3 (3.26)a
7	59.7 (3.06)a	28.7 (2.28)a	87.5 (2.67)a
14	58.0 (2.11)a	27.9 (2.94)a	85.0 (2.56)a

*M. raptorellus* released weekly for 12 wk at 13 parasitoids per bird and *N. vitripennis* released at 2.5 parasitoids per bird. Within a column, means followed by the same lower case letter are not significantly different ( $\alpha = 0.05$ ,  $df = 3$ , 1smeans/slice Tukey's multiple range test).

was highest from the 2-d exposed sentinel pupae treatment ( $F = 9.55$ ,  $df = 3$ ,  $P \leq 0.0001$ ). The interaction term exposure day \* pupal source was not significant for any of the variables.

No significant differences were observed in the successful parasitism main effects model for *N. vitripennis* ( $F = 0.71$ ,  $df = 7$ ,  $P < 0.6604$ ), *M. raptorellus* ( $F = 0.90$ ,  $df = 7$ ,  $P < 0.5172$ ) or *M. raptor* ( $F = 0.22$ ,  $df = 7$ ,  $P < 0.9771$ ). This suggests that the two pupal types and length of exposure do not impact results when sampling these three species.

Parasitoid species composition did not change appreciably as the pupae were increasingly exposed to parasitoid attack (Table 3). However, 4-d exposed pupae had a higher level of successful *N. vitripennis* parasitism than the remaining three exposure days combined. Parasitism attributed to *M. raptor* increased with duration of exposure.

This study addressed the following two issues that have concerned scientists working with pteromalid parasitoids: (1) the impact of the source of sentinel pupae and (2) the length of exposure of these pupae. Petersen and Watson (1992) and Jones and Weinzierl (1997) both suggested that field-collected pupae provide the best means to survey all parasitoid species. However, the age of these pupae are often called into question. Jones and Weinzierl (1997) attempted to select for darkened pupae that were greater than 2 d old, thus allowing for some level of parasitoid exposure. However, they suggested that their sampling was

**Table 3. Species composition of parasitoids recovered from sentinel house fly pupae exposed for various time periods in a New York high-rise, caged-layer poultry facility following release of *N. vitripennis* and *M. raptorellus***

Day's exposed	% successful parasitism		
	<i>Nasonia vitripennis</i>	<i>Muscidifurax raptorellus</i>	<i>Muscidifurax raptor</i>
2	0.5	89.5	10.0
4	2.3	86.7	11.0
7	0.9	87.4	11.7
14	0.7	87.1	12.2

*M. raptorellus* released weekly for 12 wk at 13 parasitoids per bird and *N. vitripennis* released at 2.5 parasitoids per bird.

biased by the collection of some pupae before their vulnerability to parasitism had ended. In our study, we documented that 2-d-old pupae were parasitized at a significantly lower rate than pupae exposed for 4, 7, and 14 d. Our observation that 7- and 14-d exposed pupae were also not significantly different from 4-d exposed pupae suggests that parasitism had peaked by the fourth day and that these fly pupae were probably too mature for parasitism. We found no differences between pupal source suggesting that when sampling for *Muscidifurax raptor*, *M. raptorellus* and *N. vitripennis*, pupal source is not a confounding factor.

Factors enticing parasitoids to attack naturally-occurring pupae over laboratory-reared pupae may include external pupal cues possibly obtained from the environment (Wylie 1958, Rivers 1996) and location in the manure pack (Petersen and Watson 1992, Jones and Weinzierl 1997). In our study field-collected pupae were acquired in a different manner than in previous studies (Petersen and Watson 1992, Jones and Weinzierl 1997). Collecting and returning manure to the laboratory and rearing the larvae in their breeding substrate provided for a "naturally-reared" comparison, while also controlling for the age of the pupae. However, to be employed in research, a trip to a poultry facility three days before a planned use of sentinel pupae would be required. Further, because of biosecurity concerns pupae from one farm could not be used on other farms, necessitating separate trips to each farm to be surveyed. These results validate the use of lab-reared sentinel house fly pupae in high-rise, caged-layer poultry facilities as an inexpensive, convenient and effective method to monitor populations of parasitoids.

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