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Oviposition and Development of *Fannia* spp. (Diptera: Muscidae) on Poultry Manure of Low **Moisture Levels**

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ABSTRACT Adults of Fannia canicularis (L.) and F. femoralis (Stein) were given a choice of poultry manure of 25, 35, 45, 55, and 65% moisture for oviposition. Adult F. canicularis laid most eggs on 55 and 65% moisture manure, whereas adults of F. femoralis laid most eggs on 65% moisture manure. Few eggs of either species were laid on manure ≤45% moisture. Larval development trials were conducted at 33, 40, 47, 52, and 56% manure moisture, noting number emerged, time required for emergence, and adult size. Neonate larvae of F. canicularis developed well at moisture levels \geq 47%, some developed slowly and into smaller adults at 40%, whereas 33% moisture was lethal. Manure moisture of 40-47% depressed emergence and slowed development of neonate F. femoralis larvae. Early third instars of Fannia spp. were more resistant than neonate larvae to low moisture conditions. Some third-instar F. canicularis could survive and emerge at 33% moisture, whereas some third-instar F. femoralis could emerge at moisture levels of ≥40%. Overall, F. canicularis tended to use drier manure for oviposition and could develop in lower moisture conditions relative to F. femoralis. Implications of these moisture requirements for management are discussed.

KEY WORDS Fannia, Diptera, moisture, manure, development

FLIES ARE SEVERE problems in confined poultry systems. The primary fly pests are the house fly, Musca domestica L., and the little house fly, Fannia canicularis (L.) (Axtell and Arends 1990, Axtell 1999, Hinkle and Hickle 1999). Another species, F. femoralis (Stein), also can be abundant in poultry manure (Wills and Mullens 1991, Mullens et al. 1996). Because the larvae resemble those of F. canicularis, F. femoralis may be perceived as a problem by fly inspectors, although the adults are not pestiferous.

The house fly does well in hot weather when the manure moisture range is 60-75% (Stafford and Bay 1987). Thus, M. domestica actually is easier to control in southern California than elsewhere due to usually excellent manure drying conditions in summer. In contrast, F. canicularis densities are greatest in the spring, when manure does not dry as well, and this fly has been the most common cause of fly complaints near California poultry operations for many years (Meyer and Georghiou 1987). The males hover at about eye level and are very obvious to a homeowner. Larval development of both Fannia spp. is inhibited at temperatures above 27-30°C (Meyer and Mullens 1988).

Kliewer and Boreham (1964) tested oviposition preferences of one rearing cage of adult F. canicularis over a 15-d period on poultry manure with different moisture levels. These adults laid few eggs on manure with 33% moisture and preferred manure of 50-56% moisture. In Deal (1967), emergence of F. canicularis and F. femoralis immatures was studied in a fibrous artificial medium usually used for M. domestica (CSMA media, Ralston Purina, St. Louis, MO). Development was poor below 43% moisture, good at 64-74% moisture, and poor for F. canicularis (but good for F. femoralis) at a moisture level of 82%. Anderson and Poorbaugh (1964) noted the tendency of F. canicularis to inhabit drier medium; mortality of larval F. canicularis in laboratory CSMA medium was >90% at 70% moisture, whereas mortality was <30% at moisture levels of 35 and 50%. More recently Faturochim et al. (1989) examined oviposition and larval development in poultry manure of several different fly species at a wide range of manure moisture levels (40-90%), and F. femoralis could develop in manure of lower moisture relative to other muscoid flies.

Manure often is allowed to accumulate within a poultry house for several months or longer. In the process, the manure piles dry and develop a diverse community of arthropods, leading to a generally negative relationship between manure mass and fly emergence (Legner and Bowen 1973). Even in winter, southern California poultry manure accumulating in

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this way is typically <65% moisture (Mullens et al. 1996). However, there have been observations (B.A.M., unpublished data) of significant spring Fannia spp. development in manure under the best recommended in-house manure drying practices. The current study thus focused on the effect of low-moderate manure moisture levels appropriate to the arid southwestern United States on Fannia spp. oviposition and development.

Materials and Methods

Caged-layer (white leghorn) poultry manure was obtained from surface manure accumulations (5-10 cm) on a southern California ranch that had not used pesticides recently. A large and uniform manure supply was needed for 2 yr of planned experiments. Manure was frozen (-20°C) to kill any existing arthropods and sun-dried (40-45°C) to <10% moisture. To ensure uniformity the manure then was processed through a commercial blender and sieved through a 16-mesh screen to exclude feather debris. The dry manure was placed into sealed plastic bags and stored at 4°C pending use in the experiments. In each experiment, the manure was first oven-dried at 50°C overnight to eliminate residual moisture. It was then reconstituted using deionized water to the appropriate moisture level.

Flies (F. canicularis and F. femoralis) were collected as adults on a southern California poultry ranch and colonized for use in experiments. Adults were maintained with sugar and dried milk for food, and larvae were reared on prefermented fly larval medium, consisting of wheat bran, alfalfa meal, dried milk, yeast and water. Fly rearing and laboratory experiments were conducted at a temperature of $22 \pm 2^{\circ}$ C.

Oviposition Experiments. The dry manure was weighed and reconstituted to the appropriate moisture levels using deionized water ([water weight/manure + water weight] × 100). For oviposition experiments, a series of 120-ml plastic cups was filled in the morning halfway with the reconstituted manure, which was gently compacted to form a smooth surface. Manure moisture levels were 65, 55, 45, 35, and 25%. Levels >65% were not used, because southern California manure is seldom >65% unless there are water leaks. Glass marbles (13 mm diameter) were placed in a single layer on the manure surface. This structural cue was needed for both species to oviposit, but provided a repeatable treatment surface structurally consistent across moisture levels.

A group of 50 gravid females was placed into each of four fly cages. Five cups of manure (one cup of each moisture level, placed in randomized positions in the cages) were presented for 4 h from midmorning to early afternoon (F. canicularis) or for 16 h overnight (F. femoralis). Eggs on the manure surface, or on marble and cup surfaces immediately adjacent to the manure, were counted. The entire experiment was repeated three times for F. canicularis and twice for F. femoralis. Numbers of eggs per cup were transformed to log10 (n+1) and subjected to analysis of variance

(ANOVA), followed by Tukey's honestly significant difference (HSD) to separate the means ($\alpha = 0.05$).

Larval Development and Emergence. Plastic 120-ml cups were filled with 60 ml of manure each. It was noticed in preliminary trials that adding water to manure cups to maintain a constant weight resulted in progressively wetter manure over the period required for Fannia development. Therefore we first determined the rate of dry weight loss by setting up 18 cups at each of the five moisture levels: 56, 52, 47, 40, and 33%. Based on preliminary trials, an estimated dry weight loss of ≈1% per day was assumed, and deionized water was added accordingly each day to maintain the appropriate moisture level. Three cups were removed on each of the days 1, 5, 9, 13, 17, and 21 after setup. Cups were dried completely in an oven at 50°C for 2 d, and weighed to determine dry weight loss. Weight was regressed against time, and from the slopes correction factors were generated to estimate the amount of water needed to maintain the appropriate moisture level. A similar experiment was set up including 20 early third instars of F. canicularis in each cup, except that weights were checked on days 1, 4, 8, 11, and 14. In this case approximate wet weight of the larvae (0.2 g) was subtracted before oven-drying of the manure.

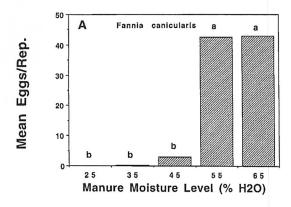
For development experiments, neonate larvae (<1 d old) were gently added in groups of 30 to the surface of manure of known moisture levels (four replications/level), using a camel's-hair brush. Cups were held within a secondary (1 liter) plastic container. These secondary containers had a lower half that held the sample; emerging flies passed upward through a funnel and were captured in the upper half. Deionized water was added every 1–2 d to maintain the appropriate moisture level during larval development. Once it appeared that all larvae had pupated, no more water was added.

The numbers of flies emerging were counted every 1-2 d. In the first trial for each Fannia species, emerged adults were saved by day and treatment in vials of 70% EtOH for later measurements of wing length, as an approximation of size. Experiments using neonate larvae were repeated three times for F. canicularis and twice for F. femoralis.

A second series of replicated experiments was done using third instars of each Fannia species. Larvae 5–7 d of age were removed from the regular Fannia larval medium and added to cups of manure (30/cup, four replications per level) and held as described above for neonate larvae. At this time they were early third instars, and would be expected to require another 4–7 d of feeding and development before pupating at 21°C (Meyer and Mullens 1988). Emerging flies were counted every 1–2 d, and both time to emergence and number emerging were analyzed using ANOVA and Tukey's HSD to separate means ($\alpha = 0.05$).

Results and Discussion

Oviposition Experiments. Manure moisture had a significant effect on oviposition by F. canicularis (F =



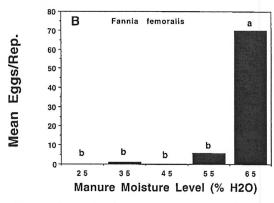


Fig. 1. Oviposition by Fannia spp. presented with a choice of poultry manure of different moisture levels. Within a species, means labeled with the same letter are not significantly different using Tukey's HSD test (P > 0.05). (A) F. canicularis. (B) F. femoralis.

24.64; df = 4, 45; P < 0.001) and F. femoralis (F = 8.70; df = 4, 30; P < 0.001). In production houses 80–90% moisture manure is nearly liquid, 55-70% manure retains some semblance of structure, and 40-50% manure consists of easily separated, discrete clumps. By using marbles as a consistent structural cue, we were able to compare low-to-moderate manure moisture levels without these being confounded by structural differences. Fannia canicularis oviposited predominantly on manure of 55% and 65% moisture; significantly fewer eggs were laid on 45%, an occasional egg on 35%, and no eggs on 25% moisture manure (Fig. 1A). Fannia femoralis laid almost all eggs on 65% moisture manure, some on 55%, and essentially none on manure of 45% or less moisture (Fig. 1B). Therefore, both species clearly preferred at least moderately moist manure for oviposition.

Direct comparisons with the study of Faturochim et al. (1989) are complicated by different methods. Manure in that study had a minimum of processing (no freezing, drying, grinding or sieving) and was thus more natural but also possibly more variable in content or structure. The current study counted eggs directly, whereas after fly oviposition the Faturochim et al. (1989) study assessed oviposition by bringing the

manure back to 75% moisture, waiting 2 d, and extracting the second instar larvae using Tullgren funnels. Despite the major methodological differences, the results show similar trends. Data from Faturochim et al. (1989) showed that most manure flies (Musca, Muscina, Hermetia, Hydrotaea, F. femoralis) prefer manure of \approx 70% moisture for oviposition, but *F. femoralis* also used drier manure. Our studies confirm the pestiferous little house fly, F. canicularis, is even more able to use relatively low moisture manure (55%) for oviposition. It can be difficult for California producers to achieve levels <55% on the surface of accumulated manure, particularly in winter and spring drying inhouse (Mullens et al. 1996). Therefore it is likely that manure suitable for oviposition exists year-round, although good drying conditions may minimize the volume of acceptable substrate.

To maintain the appropriate moisture levels, it was necessary to account for dry weight loss of the manure over time in fly development studies. In degradation tests without larvae, dry weight loss resulted in a significant negative slope in the regression (weight versus time) at every moisture level (absolute t >-3.19, P < 0.01). Daily percentage weight loss was 0.5% at 33% moisture, 1% at 40% moisture, and 1.3-1.4% at higher moisture levels. In tests with larvae, negative slopes were significant for all moisture levels above 33% (absolute t > -4.76, P < 0.001), whereas at 33% the slope was not significantly different from 0 (t =-1.54, P > 0.1). Daily dry weight loss was $\approx 1\%$. It is possible the weight loss was enhanced by the grinding of the manure, maximizing the surface:volume ratio and exposure of the manure to microbial degradation. However, these figures agree with data in Barnard et al. (1998), who documented a poultry manure loss of ≈35% dry weight over 4 wk (≈1.2% per day) in the presence of M. domestica larvae. If a study merely held manure weight constant over a 2-wk fly development period, this could amount to a cumulative error (inadvertent, gradual increase in moisture) of 15-20%. Because our loss figures are fairly close with and without fly larvae, most of the degradation is probably due to microorganisms.

Larval Development and Emergence. Manure moisture substantially affected the ability of neonate *Fannia* spp. to develop, both in terms of numbers and time required for emergence (Table 1; Fig. 2). Development of neonate *F. canicularis*, either in terms of numbers emerged or time, was stable at moisture levels ≥47%. Significantly fewer *F. canicularis* adults emerged, and emergence was significantly delayed, at 40% moisture; it took 33% moisture to prevent development completely.

A similar trend was observed for neonate *F. femoralis*, although data were analyzed by trial due to significant trial effects (Table 1). Some of the trial variability probably was caused by differences in actual larval age (e.g., neonate larvae did vary from a few hrs to slightly over 24 h old when selected). Temperatures also varied somewhat in the laboratory over time, contributing to significant differences in developmental time among experimental trials. In trial one

Table 1. Survival (n = 30/rep) and time to adult emergence of Fannia spp. larvae placed into cups of poultry manure of different noisture levels

Species	Stage	Trial	Manure moisture, %	No. emerged (mean \pm SD)	Days to adult (mean ± SD)
Fannia canicularis	L1	1-3	33	$0.0 \pm 0.0c$	NA
			40	$14.2 \pm 7.2b$	$25.5 \pm 2.5a$
			47	$23.6 \pm 3.2a$	$21.9 \pm 1.4b$
			52	$23.7 \pm 3.4a$	$22.4 \pm 1.2b$
			56	$23.8 \pm 2.7a$	$21.9 \pm 0.7b$
	L3	1	33	$2.7 \pm 1.5d$	$26.5 \pm 2.3a$
			40	$14.3 \pm 2.9c$	$21.8 \pm 1.9b$
			47	$21.3 \pm 1.5b$	$21.5 \pm 1.0b$
			52	$26.8 \pm 2.6a$	$19.6 \pm 0.4b$
			56	$25.0 \pm 1.2ab$	$19.2 \pm 1.0b$
		2	33	$22.8 \pm 2.2a$	$21.2 \pm 0.4a$
			40	$25.5 \pm 2.5a$	$18.9 \pm 0.3b$
			47	$26.8 \pm 3.0a$	$19.2 \pm 0.6b$
			52	$24.0 \pm 3.9a$	$18.8 \pm 0.7b$
			56	$21.8 \pm 0.9a$	$18.0 \pm 0.3b$
		3	33	$10.8 \pm 2.2b$	$21.6 \pm 1.9a$
			40	$21.8 \pm 3.4a$	$18.7 \pm 1.1b$
			47	$24.0 \pm 2.6a$	$18.6 \pm 0.5b$
			52	$23.5 \pm 1.3a$	$20.0 \pm 0.9ab$
			56	$24.3 \pm 2.6a$	$18.9 \pm 0.6b$
Fannia femoralis	Ll	1	33	0.0 ± 0.0 b	NA
			40	$21.5 \pm 1.7a$	$16.1 \pm 1.0a$
			47	$23.3 \pm 6.3a$	$15.0 \pm 0.1ab$
			52	$28.3 \pm 2.1a$	$14.8 \pm 0.2b$
			56	$25.3 \pm 3.6a$	$14.5 \pm 0.2b$
		2	33	$0.0 \pm 0.0c$	NA
		-	40	$2.8 \pm 3.6c$	$25.7 \pm 5.8ab$
			47	$16.8 \pm 5.1b$	$26.5 \pm 2.7a$
			52	$23.3 \pm 1.7ab$	20.8 ± 1.0 ab
			56	$27.3 \pm 3.2a$	20.3 ± 0.8b
	L3	1	33	$0.0 \pm 0.0 d$	NA NA
	2.	•	40	$13.8 \pm 5.8b$	$17.1 \pm 0.8a$
			47	29.5 ± 1.9c	$15.5 \pm 0.2b$
			52	26.5 ± 3.5c	$15.2 \pm 0.2b$
			56	28.3 ± 2.9c	$15.2 \pm 0.1b$
		2	33	0.0 ± 0.0 b	NA
		-	40	$6.3 \pm 3.1b$	$18.2 \pm 1.6a$
			47	$24.3 \pm 3.4a$	16.8 ± 0.4ab
			52	$24.5 \pm 6.6a$	$16.2 \pm 0.7b$
			56	$22.2 \pm 12.7a$	$15.8 \pm 0.3b$
		3	33	0.0 ± 0.0 b	15.6 ± 0.5b NA
		J	40	$20.7 \pm 6.0a$	$19.1 \pm 0.3a$
			40 47	$19.0 \pm 3.2a$	
			52		17.3 ± 0.6b
			52 56	$19.0 \pm 4.5a$	17.6 ± 0.9b
			90	$26.8 \pm 3.5a$	$16.5 \pm 0.6b$

Means within a species, stage and trial followed by the same letter are not significantly different using Tukey's HSD test (P > 0.05).

numbers of emerging adults were not significantly reduced until 33% moisture prevented development, although adults emerged significantly later at 40% moisture. In trial 2, emergence was significantly less at 47% moisture relative to 56%, and hardly any adults emerged at 40%. As was true for *F. canicularis*, *F. femoralis* adults also were significantly smaller at 40% moisture relative to higher moisture levels (Table 2).

Third instars varied from 5 to 7 d when added to the manure, and this probably also contributed to variability among trials. Larvae that were relatively closer to pupation, even by a day or two, would tend to be more tolerant of drier conditions. In this respect the trials present a more complete picture of variability in survival that might be expected if late stage larvae were removed from a poultry house to a manure drying pad. Despite trial variability, late instar Fannia spp. consistently were more tolerant of dry manure con-

ditions than were early instars. Results for the pooled trials are shown in Fig. 2. Late stage larvae of *F. canicularis* generally did well at moisture levels ≥40% (Table 1). Although emergence was usually reduced and delayed by drier conditions, some could complete development at 33% moisture, and emergence in trial 2 even at 33% was not reduced significantly.

Third-instar F. femoralis, in contrast, were significantly stressed at 40% moisture (reduced emergence and prolonged development time), except in trial 3. No adults emerged at 33%. In both species there was a strong negative correlation (r = -0.46 for F. femoralis and r = -0.58 for F. canicularis; P < 0.01) between number emerging and developmental time.

Despite the methodological differences noted earlier, data from Faturochim et al. (1989) also support the interpretation that *Fannia* spp. are unusually tolerant of dry manure relative to most other manure-

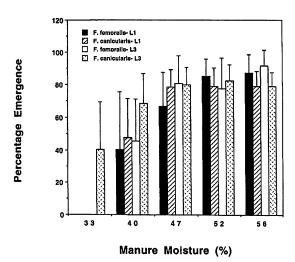


Fig. 2. Adult emergence success (mean ± SD, trials pooled) of larvae of *Fannia* spp. after placement into poultry manure of different moisture levels. Larvae were added as neonate larvae (L1) or third instars 5-7 d old (L3).

dwelling flies. Smith (1969) removed larvae of *F. canicularis* from larval media at different days of development, placing them into cups of dry sand to determine if they could successfully pupate and emerge. At 24°C and 45% RH, larvae removed as early as day 9 could pupate and emerge as adults. The Smith (1969) study also is not strictly comparable to the current study, since larvae removed to sand also were denied any further opportunity to feed, but still suggests older immatures are resistant to desiccation.

The results of the current study are not encouraging for direct suppression of Fannia spp. through the widely used technique of manure moisture reduction. Fannia femoralis seems to require somewhat higher manure moisture than does the more significant pest. F. canicularis, although even femoralis does well at fairly low moisture levels. Early stage larvae can completely develop in manure that is as dry as ever might be expected within poultry houses using a manure buildup system, particularly in spring. Once larvae have hatched, accumulated manure should have adequate moisture to support complete development. Manure removed from poultry houses and spread on drying pads in a 2.5 cm thick layer was reduced from 69 to 10% moisture in 48 h under southern California winter conditions, with about the same moisture re-

Table 2. Wing lengths in mm (mean ± SD) of Fannia spp. emerging from poultry manure of different moisture levels

Moisture, %	F. canicularis	F. femoralis
40	$3.84 \pm 0.21b$	2.73 ± 0.15 b
47	$4.19 \pm 0.20a$	$2.93 \pm 0.08a$
52	$4.28 \pm 0.15a$	$2.96 \pm 0.06a$
56	$4.35 \pm 0.05a$	$2.95 \pm 0.09ab$

Within a species, means labeled with the same letter are not significantly different using Tukey's HSD test (P > 0.05).

duction in 24 h in summer (Fairbank et al. 1988). Properly spread manure in areas such as southern California thus should still prevent completion of development by Fannia spp, particularly if temperatures exceed 27–30°C (Meyer and Mullens 1988). Studies on the fate of Fannia larvae in manure removed to a drying pad and spread in thicker layers, however, would be worthwhile, because it is common for manure not to be spread as thinly as 2.5 cm.

These studies suggest that it is probably unusual for poultry manure accumulating in-house to be dry enough to deter oviposition by Fannia spp. significantly in winter or spring, and once larvae are present, there certainly should be enough moisture to allow them to develop. Despite this finding, a dry manure pad, left as a base after a manure cleanout, elevates the manure and encourages drying (Mullens et al. 1996), and the pad is known to be advantageous for Fannia control (Legner and Bowen 1973, Meyer et al. 1987). The mechanism may be encouragement of natural predators and parasites, which are more abundant and are thought to forage more efficiently in stable, drier manure conditions (Legner and Dietrick 1974, Legner et al. 1975).

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