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EFFECT OF FLUORESCENT POWDER ON *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE) AND A SIMPLE DEVICE FOR MARKING SAND FLIES

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ABSTRACT. A simple device for marking phlebotomine sand flies with fluorescent powders is described and tested; the design of the new device is characterized by separate compartments for sand flies and powder. The effect of fluorescent powder on survival and mobility of the sand fly *Lutzomyia longipalpis* was compared using our device (method A) and a single-container method (method B). Mortality within 1 h of powder application was negligible for method A (0.9%), but was 19.8% for method B; in addition, method B also reduced sand fly mobility. Adherence of excess powder to the sand flies observed with method B was responsible for the negative effects observed during the marking process. In field releases, however, recapture rates were the same for each method. Neither sand fly mobility or survival were adversely affected if appropriate quantities (method A) of fluorescent powder were applied to the exoskeleton of these insects.

INTRODUCTION

The use of fluorescent powders to mark phlebotomines has been limited and as a result the effects of these powders on sand fly survival and behavior are poorly understood. Studies on dispersal, longevity, gonotrophic cycle length, mating, and aggregation behavior have suggested that these methods have no deleterious side effects (Quate 1964, Foster 1972, Chaniotis et al. 1974, Killick-Kendrick et al. 1984, Doha et al. 1991, Dye et al. 1991, Alexander and Young 1992, Jarvis and Rutledge 1992), or that mortality is limited to the marking process itself (Morrison et al. 1993).

To date, published methods for applying fluorescent powders to the exoskeleton of phlebotomines can be divided into 2 general categories. In the first, sand flies are confined to a receptacle containing a small quantity of powder; the dust is then agitated by blowing air into the container (Alexander 1987). In contrast, the other technique keeps the flies separated from the powders that are sprinkled over the flies prior to their release (Rioux et al. 1979).

The objectives of the present study were to evaluate the consequences of marking the sand fly *Lutzomyia longipalpis* (Lutz and Neiva) with fluorescent powders. We developed a new marking device that prevents sand flies from physical contact with powder in the base of the container, but allows for the application of larger quantities of powder than methods that sprinkle dust over the flies. The marking efficiency, and effects on sand fly mobility, mortality, and longevity were evaluated for our device and are compared with another marking technique (Alexander 1987).

MATERIALS AND METHODS

The 2 marking techniques were compared in both field and laboratory experiments. All field experiments used *L. longipalpis* of unknown age, captured in El Callejón (4°18′N, 74°42′W), Municipality of Ricaute, Department of Cundinamarca, Colombia. El Callejón is a known endemic focus of American visceral leishmaniasis (AVL) (Corredor et al. 1989). *Lutzomyia longipalpis* is the predominant sand fly species in the community, accounting for more than 98% of the sand flies captured in animal bait (pigs and cattle) collections (Morrison et al. 1993, Ferro et al. 1995). This site has been described in detail by Ferro et al. (1995). Laboratory experiments described below used 1–3-day-old sand flies from a *L. longipalpis* colony that originated from El Callejón. The colony was maintained at the National Institute of Health (INS) in Santa Fe de Bogotá, Colombia, using the techniques of Modi and Tesh (1983).

Marking apparatus: Our marking device was designed to protect the sand flies from the potential physical damage associated with resting in fluorescent powder. Insecticide susceptibility test containers were used to construct the device (World Health Organization 1970), which contained 4 parts: a plastic container for the flies (Fig. 1A), a smaller container for the fluorescent powder (Fig. 1C), a connector containing a slid-

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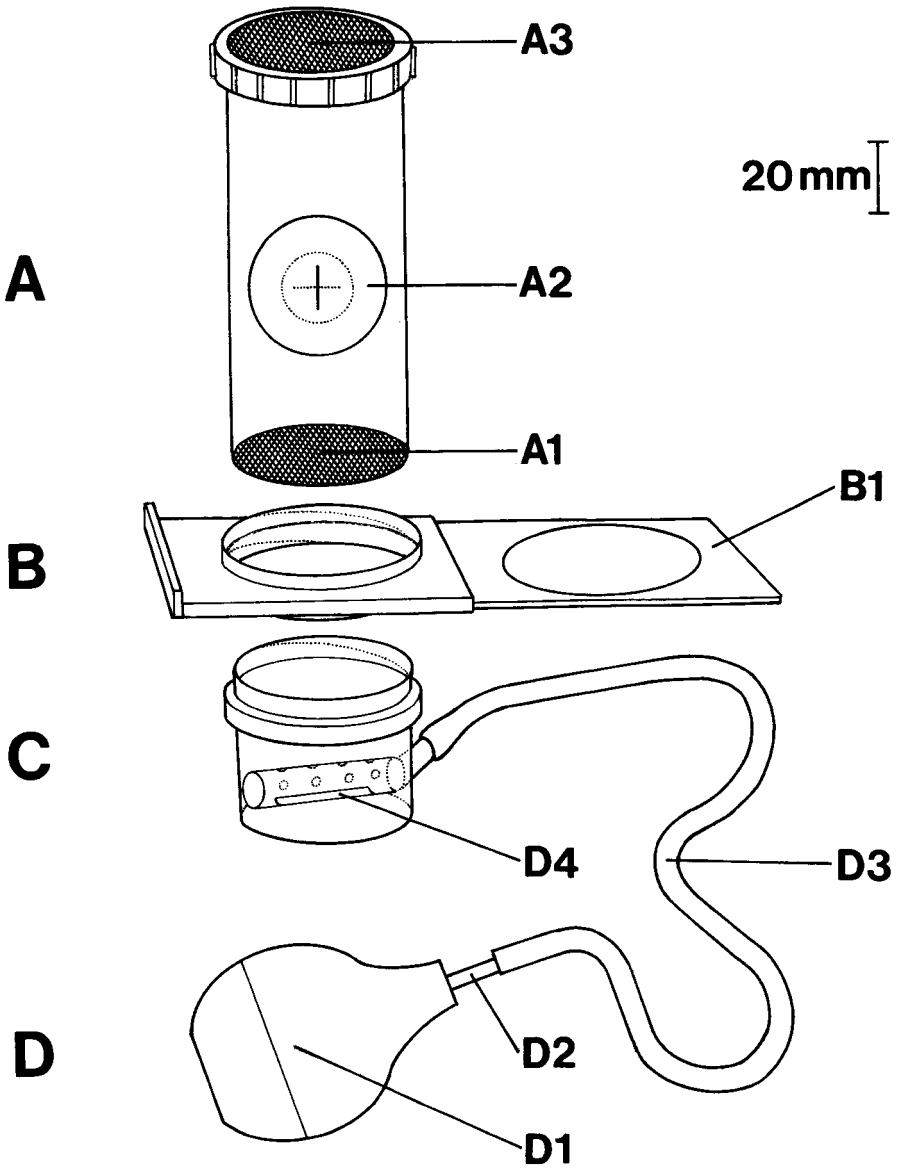

Fig. 1. Device for marking phlebotomine sand flies with fluorescent powders. A: Insect compartment; A.1: plastic screen; A.2: entrance for sand flies; A.3: screw top. B. Connector; B.1: sliding door. C. Container for powder. D. Air pump; D.1: bulb; D.2: connector tube; D.3: rubber tubing; D.4: air distribution tube.

ing door between the 2 compartments (Fig. 1B), and an air tube connecting a bulb to the lower compartment (Fig. 1D). Sand flies were confined in a plastic container (125 × 42-mm diam) with a screw top (Fig. 1A.3); a plastic screen (0.12 mm^2) (Fig. 1A.1) in the base of the container prevented physical contact between the sand flies and the layer of powder in the lower compartment (Fig. 1C). The insect container also had a 25-mm opening on its side; 2 layers of latex with perpendicular cuts (Fig. 1A.2) provide an entrance for the sand flies (Endris et al. 1982). The screw top contained a hole (38-mm diam) covered with very fine plastic screen (Fig. 1A.3); this top was removed to liberate the marked flies.

The powder (lower) compartment is 50 × 42-mm diam with a threaded top (Fig. 1C). The adapter (Fig. 1B) attaches to the lower end of the insect container and the upper end of the

powder compartment. The middle portion of the adaptor has a thin layer of plastic (142 × 59 mm), containing a hole (47-mm diam) that acts as a sliding door (Fig. 1B.1); when the hole is lined up with the adaptor, powder can be blown into the compartment containing the flies (open), whereas when the door is pulled outward the 2 compartments are separated (closed).

A pump forces a fine aerosol of powder from the lower to the upper compartment (Fig. 1D); the pump consists of a bulb (55-mm diam) (Fig. 1D.1) connected to 570 mm of 6-mm rubber tubing (Fig. 1D.3) by a 50 × 6-mm plastic tube (Fig. 1D.2), and small plastic tube (6-mm diam) placed 7 mm above the base of the lower compartment (Fig. 1D.4). The former tube distributes the powder uniformly through the container, blowing air through 13 holes (2.5-mm diam) distributed in 3 rows on the top and sides of the tube; in addition, the lower surface of the tube contains a 30 × 2.5-mm slot (Fig. 1D.4); one end of this piece contains a 25-mm-long tube at a 45° angle that connects to the rest of the air pump system.

Marking techniques: For each experiment described below, 2 groups of *L. longipalpis* were marked simultaneously: one with the apparatus described above (method A) and the second group with a marking method that allows the sand flies to make contact with the powder in the container (method B) (Alexander 1987). Before confinement in the appropriate container, *L. longipalpis* from either the colony or field were counted in narrow-mouth aspirators. The proportion of female to male sand flies was ≈1:1 for laboratory experiments, whereas for field experiments the ratio was ≈1 : 3.5.

Method A. Sand flies were placed in the upper compartment of the device described above with a 5-mm layer of powder in the lower compartment. Both compartments were then attached to the adaptor with the sliding door closed. To mark the insects the sliding door was opened and the bulb was pumped continuously for 1 min, producing a weak air current that carried the powder into the upper compartment. Finally, the sliding door was closed and the container carried to the chosen release site.

Method B. Sand flies were released into large plastic vials (108 × 67-mm diam) containing a 2–3-mm layer of fluorescent powder. The powder was agitated by blowing in the side of the container 3 times with a narrow mouth aspirator.

Experiment 1: A group of approximately 60 *L. longipalpis* was marked with each method (A and B), using red powder (93068, Lawter International Inc., IL). Immediately following powder application, the marking containers were placed in separate cloth cages for 1 h with their screw tops removed. The surviving flies were then killed with chloroform and examined along with the flies that died in the container. Using a stereoscope, the distribution of powder over the fly's body was evaluated qualitatively by 2 categories: sufficient and excessive. A designation of sufficient was assigned to a fly when powder covered 30–100% of its body surface; powder coverage was scored as excessive when 100% of the body surface had powder at a thickness that obscured the exoskeleton on ≥30% of the fly's body. The proportion of surviving sand flies abandoning each container after 1 h was compared to that of control groups of sand flies confined to containers without powder. Six replicates for each method were carried out in July and December, 1992.

Experiment 2 (field trials): A series of mark–release–recapture experiments was conducted to evaluate the effects of fluorescent powders on dispersal of wild-caught *L. longipalpis.* Sand flies were collected between 1830 and 2000 h in a pigpen described by Morrison et al. (1995) on the same night they were released. Experiments were conducted in January, February, May, and June, 1992. The captured insects were immediately counted, sexes were identified, and the flies were placed in the appropriate marking container. Groups were marked with red and blue powders for methods A and B, respectively; for each subsequent release the powder colors were reversed. The number of sand flies ranged from 131 to 482, but the maximum number of flies marked per container was 300. Field experiments were conducted a minimum of 15 days apart. Both groups were released at the collection site by 2020 h. During the remainder of the evening, 10-min collections were begun on the half-hour of each hour.

After each 10-min collection, the captured insects were anesthetized with chloroform, shaken into a white enamel pan, and examined with an ultraviolet light. Flies that were marked with fluorescent powder then were separated and stored dry for species and sex identification.

Experiment 3: To determine the long term effects of fluorescent powders on *L. longipalpis* longevity, daily survival rates were compared between 2 groups of sand flies marked with method A: one marked with red powder and a control group. Flies marked with method B were not studied because most of the flies died within 2 days of labeling. Six replicates (n = 47–92) were carried out during January–February and May–June, 1993. After marking the flies, each group was released into a cloth cage (19 × 19 × 19 cm). After 2 days, the sand flies in each cage were offered a blood meal on an anesthetized hamster (40 min). The marked and control

Table 1. Mortality of *Lutzomyia longipalpis* within 1 h of fluorescent powder application using 2 marking methods.

	Method A			Method B			Control A		
	No. marked (dead)		Mortality	No. marked (dead)		Mortality	No. marked (dead)		Mortality
Trial	♀	♂	%	♀	♂	%	♀	♂	%
1[1]	30 (3)	29 (0)	5.1	29 (4)	32 (7)	9.8	25 (0)	33 (0)	0.0
2[2]	29 (0)	30 (0)	0.0	29 (10)	29 (11)	36.2	41 (0)	21 (0)	0.0
3[2]	22 (0)	32 (0)	0.0	33 (7)	39 (3)	16.1	41 (0)	20 (0)	0.0
4[2]	29 (0)	31 (0)	0.0	31 (12)	33 (9)	32.8	38 (0)	22 (0)	0.0
5[2]	38 (0)	21 (0)	0.0	34 (4)	23 (6)	17.5	30 (0)	30 (0)	0.0
6[3]	27 (0)	30 (0)	0.0	30 (1)	31 (0)	1.6	30 (0)	30 (0)	0.0
Total	175 (3)	173 (0)	0.9	186 (38)	187 (36)	19.8	205 (0)	156 (0)	0.0

[1] A vs. B, not significant; A and B versus controls, $P < 0.0001$.
[2] Mortality B > other groups, $P < 0.0001$.
[3] Mortality B = other groups, $P > 0.20$.

groups were each divided into oviposition containers described by Modi and Tesh (1983): one contained bloodfed females plus half of the males from the original cage (group 1) and the other consisted of females that did not feed and 50% of the males from the original cage (group 2). The oviposition containers were changed daily and the dead sand flies removed. The sand flies were maintained at a temperature of 23°C and RH of 95.4%, and provided with a saturated sucrose solution.

Statistical analysis: Chi-square tests were used to compare marking efficiency, mortality and recapture rates, and survival rates between methods A and B. The proportion of sand flies abandoning their containers were analyzed with analysis of variance; comparison of means were made using Tukey test (SAS Institute 1988).

RESULTS

Marking apparatus: The design of our marking apparatus (Fig. 1) had the following advantages: 1) the period of time the sand flies were in contact with the fluorescent powder was limited to the 1-min period when the powder was pumped to the upper compartment of the apparatus; in contrast, sand flies marked by method B were in direct contact with the powder for much longer periods of time, resulting in a higher proportion of excessively marked insects; 2) releasing sand flies from the new apparatus was easier and facilitated removal of all the insects inside, whereas with method B the powder in the container did not permit inversion or agitation of the container to remove the marked flies; 3) large numbers of sand flies could be marked in a short period of time using one powder compartment because the compartments containing sand flies were interchangeable; and 4) finally, the new apparatus was easy to use and construct and was economical ($4.00).

Experiment 1: Mortality occurring within 1 h of the marking process was consistently higher for sand flies marked with method B than either method A or both the control groups (Table 1). Overall, the 0.9% mortality associated with method A was nearly the same as for the method A control group ($\chi^2 = 1.41$, df = 1, $P = 0.23$), but significantly lower than the 19.8% observed with method B ($\chi^2 = 64.28$, df = 1, $P < 0.0001$). Mortality rates were about the same for male and female sand flies ($\chi^2 = 64.30$, df = 1, $P < 0.0001$) (Table 1).

Marking efficiency: Our device (method A) marked all the *L. longipalpis* examined ($n = 345$) with sufficient but not excess amounts of fluorescent powder. In contrast, from 20 to 98% of the *L. longipalpis* marked with method B ($n = 294$) had excess powder; the rest of the marked flies (41.5%) contained a sufficient quantity of powder ($\chi^2 = 321$, df = 6, $P < 0.0001$). Of the *L. longipalpis* marked with method B, mortality was 2.4% ($n = 125$) in sand flies covered with sufficient powder, compared to 29.7% ($n = 238$) in those that had excess powder ($\chi^2 = 34.6$, df = 1, $P < 0.0001$). Of the flies that died within 1 h of powder application, 94.7% of the females ($n = 38$) and 96.8% of males ($n = 31$) had excess powder. Method A marked male and female sand flies equally well. For method B, 52.7% ($n = 77$) of males and 64.2% ($n = 95$) of females had excess powder, suggesting that the fluorescent powder adhered more readily to females than male sand flies ($\chi^2 = 3.97$, df = 1, $P < 0.05$).

Mobility: The mean percentage of sand flies leaving their marking containers after 1 h was

Table 2. Recapture rates for field-caught *Lutzomyia longipalpis* marked with fluorescent powders using 2 marking methods.

Method	*n*	No. marked (surviving)			% recaptured (*n*)		
		♀	♂	Total	♀	♂	Total
A	4	262 (249)	921 (892)	1,183 (1,141)	2.4 (6)	7.1 (63)	6.1 (69)
B	4	267 (168)	906 (660)	1,173 (828)	6.0 (10)	6.2 (41)	6.2 (51)

much higher for flies marked with method A (63.4%) than for those marked with method B (24.6%) (ANOVA, $F = 5.29$, df = 3, $P = 0.008$). In contrast, the mean percentage of control group sand flies leaving containers was 62.2% and 75.3% for method A and B, respectively. Of the 2 treatment and control groups of sand flies, only those marked using method B showed a significant decrease in the number of sand flies abandoning their containers after 1 h (Tukey, $P < 0.05$).

Experiment 2: In 4 field experiments, a total of 1,141 and 828 sand flies were marked and released with method A and B, respectively. The overall recapture rates did not differ significantly between the 2 methods; however, the recapture rate for female sand flies marked with method A was significantly lower than for males ($\chi^2 = 7.42$, df = 1, $P = 0.007$) (Table 2). In contrast, the recapture rates for the females (6.0%) and males (6.2%) marked with method B were the same.

Overall, mortality rates for both methods were higher in field experiments than in laboratory experiments (method A: $\chi^2 = 6.81$, df = 1, $P = 0.0009$; method B: $\chi^2 = 115.24$, df = 1, $P < 0.0001$). The percentage of sand flies that died within the marking apparatus was much higher for method B (29.4%) than for method A (3.6%) ($\chi^2 = 287$, df = 1, $P < 0.0001$). The mortality for male and female sand flies was about the same for method A; in contrast, for method B, mortality in females (37.1%) was significantly higher than for males (27.1%) ($\chi^2 = 9.79$, df = 1, $P = 0.002$).

Experiment 3: Daily survival rates were monitored for *L. longipalpis* marked with method A and B compared to control groups. All of the *L. longipalpis* marked with method B died within 2 days of treatment and thus were not offered blood meals. A total of 743 *L. longipalpis* were marked with method A: 549 bloodfed and 194 did not feed. For the control groups, 545 sand flies bloodfed, whereas 196 did not. The mean survival of female *L. longipalpis* that had bloodfed was 10.1 days (Fig. 2); the treatment and control groups showed no significant difference in survival. In contrast, for the unfed female flies, the control sand flies had a significantly higher mean survival rate (17.0 days) than the treatment group (15.0 days) ($\chi^2 = 21.07$, df = 6, $P = 0.002$) (Fig. 2). Survival of male sand flies from the cage with bloodfed females was 12.1 days (Fig. 3) for both the treatment and control groups, whereas survival of the males from the cage with unfed females was 13.5 days (Fig. 3).

DISCUSSION

We have designed an apparatus that effectively marks phlebotomine sand flies with fluorescent powders. The marking device (method A) results in an evenly marked group of sand flies with a quantity a powder sufficient for easy detection with an ultraviolet lamp. The major advantage of our apparatus is that it keeps the sand flies separated from the fluorescent powder except during the actual marking process, and thus prevents the insects from being buried in a layer of powder in the base of the marking container. As a result, sand fly mortality is reduced and there is no effect on mobility. Morphological differences between the sexes are probably responsible for increased fluorescent powder adherence and thus higher mortality in female sand flies. Variation in powder adherence to other insect species has been attributed to species differences in morphological characters (Stern and Mueller 1968).

Sand fly mobility was more than 2-fold greater for flies marked with method A than with method B. The reduced mobility observed in sand flies marked with method B corresponded to a higher proportion of sand flies with excess powder. Thus, an excess of fluorescent powder should affect normal flight behavior (Akey 1991) and appears to be the primary cause of reduced mobility and mortality in the sand flies marked with method B.

When both marking methods were compared using wild-caught *L. longipalpis,* mortality associated with the marking process, as with laboratory tests, was considerably lower with our apparatus (method A). The mortality observed with method B (29.4%) was consistent with mortality rates attributed to insect manipulation in previous studies (Morrison et al. 1993). If

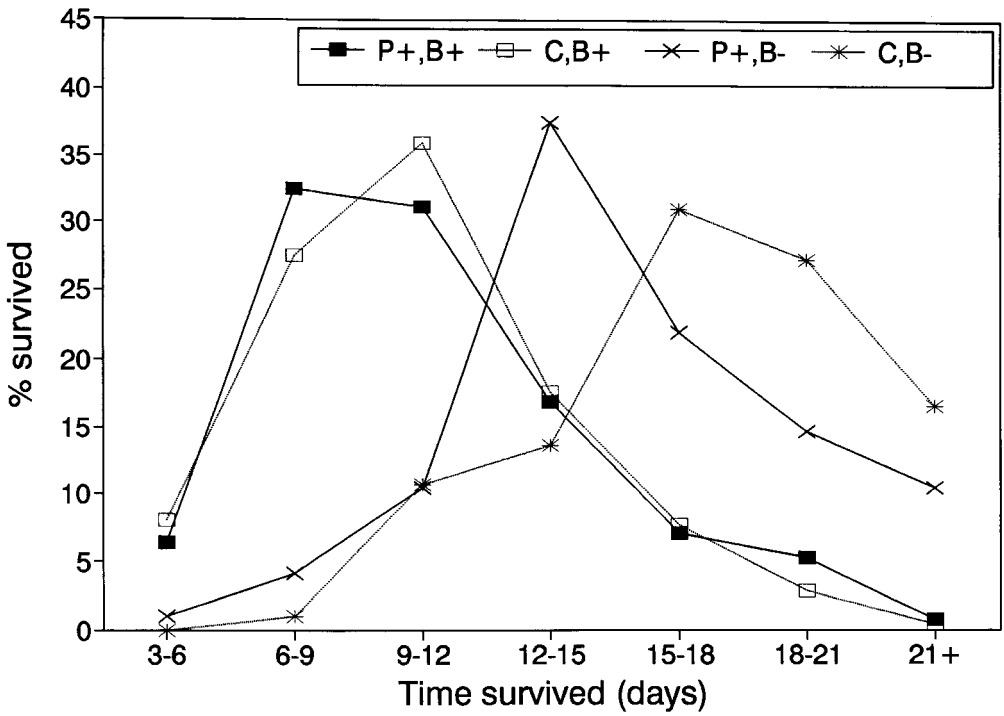

Fig. 2. Effect of fluorescent powders applied with method A on the longevity of female *Lutzomyia longipalpis*. P+, marked flies (powder-positive); C, control flies; B+, bloodfed; B−, unfed.

only considering the insects that survived the marking process, sand fly recapture rates for the 2 methods were comparable, even though flies marked with method B had reduced mobility in laboratory experiments. This discrepancy may be a result of different adherence properties of the 2 colors of fluorescent powders in our field releases (Stern and Mueller 1968, Lillie et al. 1981). If the blue powder used in this study adhered less effectively to *Lutzomyia,* then the percentage of flies with excess powder would be less than for those marked with red dust. Therefore, negative effects associated with method B could be reduced if blue powder were used. These results may also indicate that flies surviving the marking process, independent of the method employed to mark them, do not suffer long-term effects from the powder itself. In previous mark–release–recapture studies in El Callejón, daily mortality rates of marked and control sand flies were similar the 2nd through 6th day after release (Morrison et al. 1993). Unfortunately, using recapture rates to evaluate the effectiveness of the 2 methods may not be appropriate. It is possible that the most mobile flies dispersed to other sites. In previous mark–release–recapture studies, between 50 (Morrison et al. 1993) and 80% (Dye et al. 1991) of recaptured *L. longipalpis* were found within 50 m of the release site. In addition, recapture rates for female *L. longipalpis* were significantly lower than for males (Dye et al. 1991, Morrison et al. 1993). In the current studies, the recapture rate of male and female sand flies marked with method B were nearly equal (6%), whereas with method A the recapture rate for males (7.1%) was nearly 3-fold higher than for females (2.4%). The reduced mobility of flies and the higher probability that females were marked with excess powder by method B may have resulted in reduced dispersal overall, resulting in the unusually high recapture rate of females marked with method B.

Overall, our results indicate that fluorescent powders do not have long-term negative effects on sand fly survival, an observation noted by other investigators (Quate 1968, Chaniotis et al. 1974, Morrison et al. 1993). With the exception of bloodfed females, in which marked flies survived 2 days less than unmarked controls, *L. longipalpis* longevity was consistent with previous laboratory studies (Sherlock and Sherlock 1959, Killick-Kendrick et al. 1977). Negative effects of fluorescent powders are associated with the marking process itself: excess powder or physical trauma. Our apparatus reduces mortal-

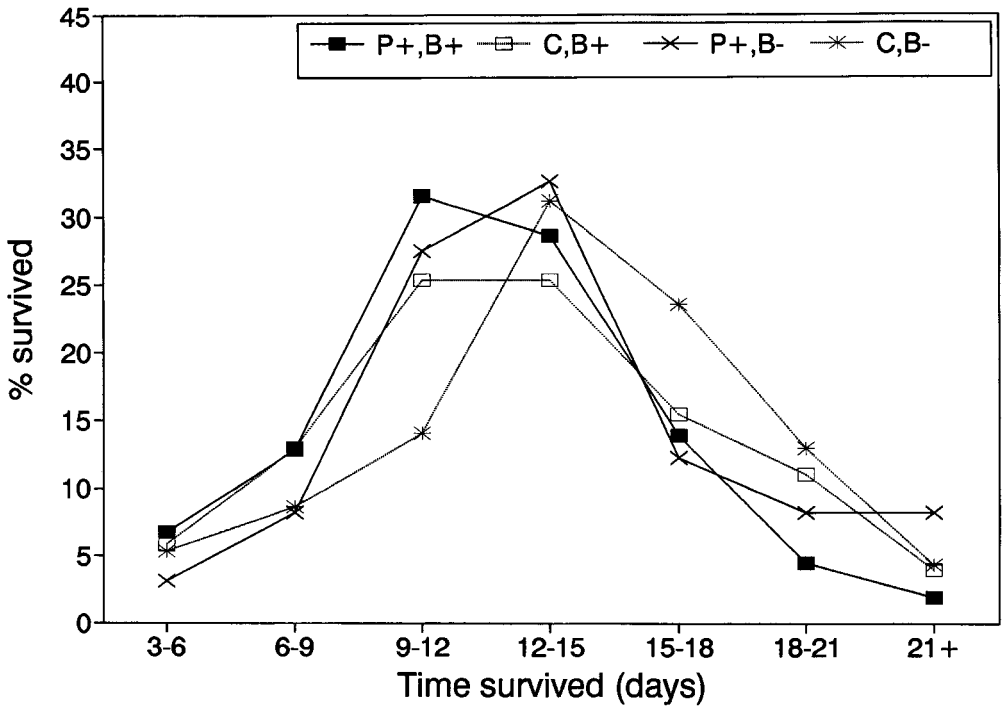

Fig. 3. Effect of fluorescent powders applied with method A on the longevity of male *Lutzomyia longipalpis*. P+, marked flies (powder-positive); C, control flies; B+, bloodfed; B−, unfed.

ity due to both of these processes. The excess mortality observed in female sand flies marked with method B is directly attributable to the observation that females were more likely to take on excess powder. The mortal effects of excess powder have been observed for other insects (Gangwere et al. 1964).

Mortality was always greater during field experiments than in laboratory experiments. This was probably due to the differences in the laboratory and field-caught *L. longipalpis*. The field-caught flies were of unknown age and nutrition. In addition, in general, a higher number of insects were released in the field experiments, resulting in the flies spending more time in their respective containers, often with more insects than their laboratory counterparts.

In summary, fluorescent powders, applied in appropriate quantities to the exoskeleton of sand flies do not effect the mobility, mortality, or longevity of the insect. An excessive quantity of powder, however, results in decreased mobility and increased mortality immediately after the marking process, and these negative effects are especially apparent in female sand flies, which appear to have increased adherence to the powders. We have introduced a design for a marking apparatus that prevents the direct contact of sand flies with fluorescent powder in the base of a marking container, and thus avoids the adherence of excess powder.

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