

Refugia Preferences by the Spiders *Loxosceles reclusa* and *Loxosceles laeta* (Araneae: Sicariidae)

RICHARD S. VETTER¹ AND MICHAEL K. RUST

Department of Entomology, University of California, Riverside, CA 92521

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ABSTRACT A variety of refugia were offered to different instars of brown recluse spiders, *Loxosceles reclusa* Gertsch and Mulaik, and a South American recluse spider, *L. laeta* (Nicolet), to determine whether they preferred certain types of refugia spaces. Variables included (1) crevice widths ranging from 3.2 to 21 mm, (2) horizontal and vertical orientations, and (3) new refugia or refugia that had silk deposited by a previous conspecific resident. An additional 30-d assay with similar-sized refugia studied each species' propensity for site fidelity or movement among refugia. *L. reclusa* preferred crevice widths ≥ 9 mm with no correlation of body size to crevice width, whereas *L. laeta* preferred crevice sizes ≥ 6.4 mm with a marginally significant correlation between crevice width and body size. Both species preferred (1) vertical instead of horizontal-oriented refugia and (2) refugia with conspecific silk compared with previously uninhabited refugia. There was no significant difference between the species in their propensity to move among refugia in the 30-d trial; however, both species had individuals that were always found in the same refugium for the entire assay and individuals changing refugia every 2–3 d. The propensity to switch refugia was not affected by the degree of starvation for the period tested as was initially hypothesized. The possible implications of this research toward developing novel control measures for *Loxosceles* spiders are discussed.

KEY WORDS brown recluse spider, *Loxosceles*, Arachnida, spider control, urban entomology

Loxosceles spiders can cause medically important skin lesions in humans. In North America, the brown recluse spider, *Loxosceles reclusa* Gertsch and Mulaik, is found almost exclusively in the central and southern midwestern states (Gertsch and Ennik 1983, Swanson and Vetter 2005). In South America, *Loxosceles laeta* (Nicolet) is found in several countries and is the most widely distributed *Loxosceles* species there (Gertsch 1967). Both *L. reclusa* and *L. laeta* are most commonly implicated in skin lesions on their respective continents (Swanson and Vetter 2006), despite the fact that dozens of additional *Loxosceles* species are known in the western hemisphere. Many of these other species live in remote areas or are rarely encountered and hence they do not interact with humans often. Both *L. reclusa* and *L. laeta* are synanthropes, which can be found in high numbers in association with human structures (Schenone et al. 1970, Vetter and Barger 2002, Sandidge 2004, Holper 2007). Their medical importance coupled with heightened awareness (and often hyperbole) by the general public translates into the need or desire to reduce or control spider populations in structures where interaction with humans is highly probable.

In North America, the genus of *Loxosceles* spiders is known by the colloquial name of recluse spiders be-

cause of their propensity to hide in dark, undisturbed areas of homes. In mornings after free-roaming periods, these nocturnal spiders may seek refuge in shoes or in items of clothing left on the floor. A typical bite scenario is a last-chance defensive act when a human presses a spider against flesh and is bitten while dressing or putting on shoes. However, another consideration regarding behavior affecting a control strategy is that *Loxosceles* spiders often exhibit site fidelity where several shed exuvia of increasing sizes are often found within a few centimeters of each other along with the spider (Fischer and Vasconcellos-Neto 2005, R.S.V., unpublished data), which might signify that some spiders do not roam frequently and, hence, might not readily encounter pest control devices. Therefore, both of these behaviors need to be considered when envisioning control strategies.

Loxosceles spiders provide a vexing problem for the pest control industry because they are difficult to eliminate from structures (Sandidge and Hopwood 2005), although control does seem attainable (Holper 2007). One possible step toward developing a control strategy is to construct a device that would be attractive as a diurnal refuge. *Loxosceles* spiders prefer dark, tight crevices such as under the flaps of cardboard boxes, behind picture frames, under furniture or piles of clothes, etc. (Hite et al. 1966, Schenone et al. 1970, Sandidge and Hopwood 2005, R.S.V., unpublished

¹ Corresponding author, e-mail: rick.vetter@ucr.edu.

data). Once the attractive physical aspects of a refuge are determined for *Loxosceles* spiders, additional control measures will be studied such as treatment of the artificial refugia with a pesticide. The objective of this study was to determine if *L. reclusa* and *L. laeta* spiders preferred certain sizes or orientation of refugia and previously occupied refugia.

Materials and Methods

Spiders. Specimens of *L. reclusa* were collected from Lenexa, KS (Johnson County), from property that was described in a previous *L. reclusa* study (Vetter and Barger 2002). The *L. laeta* colony was started from spiders collected from San Gabriel, CA (Los Angeles County), across the street from the historic San Gabriel Mission, which was a 1967 collection locale for this species listed in the genus revision of Gertsch and Ennik (1983). The *L. laeta* specimens used in this study were originally collected from this site or were reared from two already-inseminated females from the initial collection.

Spiders were maintained in individual plastic vials, the size of which correlated to the spider's size, lined half its circumference with paper toweling (to give them a substrate for silk attachment and a purchase for molting). Before being used in experiments, spiders were fed on various insects, most commonly house crickets, *Acheta domestica* L., and larvae and adults of Indian meal moths, *Plodia interpunctella* (Hübner); prey was offered sporadically from 1 wk to 2 mo. *Loxosceles* spiders are well known for surviving long periods (i.e., 6 mo) without food, although males are less able to do so than females. Newly emerged spiderlings were separated from adult females and placed into a communal 4-liter plastic jar with 40-mesh brass screen covering a ventilation hole in the plastic lid; separate jars were established for each species. Crumpled paper towels in the jar provided many refugia for the small spiderlings. Fruit flies, *Drosophila* spp., were added to the jar at sporadic intervals. We did not provide water because it is not needed for survival; fluids are obtained from feeding. *Loxosceles* spiders are very tolerant of conspecifics as long as there is sufficient prey. When spiderlings were about half-grown, they were removed to plastic vials and reared individually. Despite their potentially dangerous nature, neither species showed any propensity to bite even when running along exposed human forearms during transfer to vials, although this latter behavior is not necessarily encouraged.

Chipboard Refugia. Pieces of chipboard, i.e., the gray hardboard on the backs of notepads, (0.77 mm thick, 26670; Papermart, Los Angeles, CA), were cut to provide refugia in 50-mm widths with a length equal to 75 mm plus the desired crevice dimension. A score line, approximately half-way through the chipboard's thickness, was made at the 50-mm length with a knife blade, and a second score line was made a distance away from the 50-mm line equal to the desired crevice width. The chipboard piece was folded along each of the two score marks such that a 25-mm long wooden spacer could be affixed

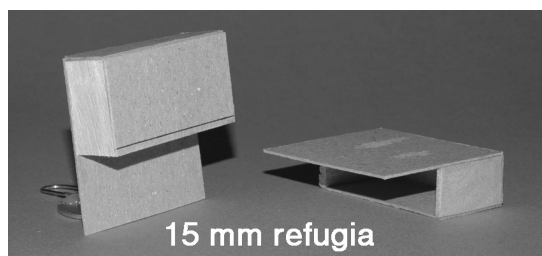


Fig. 1. Refugia constructed from chipboard and wooden spacers. Shown here are refugia with 15-mm crevices in a vertical and horizontal orientation.

on each side with Elmer's Glue-All multi-purpose glue (Elmer's Products, Columbus, OH), creating a refugium with a 50-mm backing and a 25-mm-deep crevice (Fig. 1). Crevice width varied depending on the availability of sizes of wooden materials that acted as spacers; the smallest sizes were purposely chosen to exclude the largest spiders but be sufficient for the smallest instars. For the small crevice series, the spacers were made of basswood (i.e., balsa wood) to create crevices of 3.2-, 4.8-, 6.4-, and 9-mm widths. For the large crevice series, sturdier wooden sticks (ice cream sticks, craft sticks, tongue depressors, plant stakes) of 9-, 15-, 18-, and 21-mm width were cut to serve as spacers.

General Experimental Procedure. For the vertical orientation, a 46-mm-long paper clip (72500; Acco, Lincolnshire, IL) was bent at a 90° angle and taped to the back of the 50-mm side of the refugium (Fig. 1). For the larger series (9–21 mm), to increase stability, a metal washer (32 mm diameter, 7 mm center hole, 8.7 g) was attached to the paper clip with hot glue several days before testing. The opening of the refugium in vertical orientation was always downward. Refugia were placed in round, clear polystyrene containers (185 mm diameter by 76 mm height) against the arena wall at 90° spaced intervals (or 180° if only two refugia were being compared) such that if a spider walked along the wall, it would be forced to encounter a refugium (Fig. 2). For the horizontal orientation (Fig. 1, right), the refugium

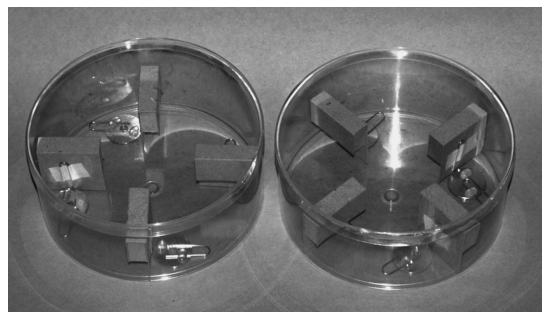


Fig. 2. Two sample arenas with different arrangements of vertically oriented refugia. (Left) The refugia crevices open toward the clockwise direction and are oriented to the four major compass points. (Right) Crevices open in a counter-clockwise direction and are angled 45° from the four major compass points.

was placed as close to the wall as possible with two corners touching the wall and the 50-mm length on top, acting as a shade to provide more shadow to the cavity. In either orientation in any particular arena, the openings of the crevices faced in the same direction (i.e., clockwise or counterclockwise) for all refugia (Fig. 2), which were randomly arranged. The arenas were placed on a gray countertop in a room exposed to west-facing windows shielded with blinds or brown wrapping paper. One half of the arenas were placed on the counter top with the refugia aligned at the major compass points (north, south, east, and west; Fig. 2, left); the other arenas had the refugia aligned 45° from the major compass points (Fig. 2, right). The orientation of the refugia openings and orientation to compass points was set up such that the order of the refugia in an arena changed from the adjacent arena in a repeated pattern of four arrangements: compass points/clockwise, angled 45°/clockwise, compass points/counterclockwise, angled 45°/counterclockwise (the first and last shown in Fig. 2). Arrangement in alternating fashion was done to reduce potential effects of environmental differences such as lighting and temperature.

Initial experiments were performed with *L. laeta* spiders; procedures were modified to increase efficiency or eliminate superfluous aspects as the results of the experiments unfolded. In preliminary experiments, it was determined that (1) checking spiders once in the morning was sufficient (instead of twice daily readings) because they did not move around the arenas during the day, (2) spiders should be set up at twilight so that they do not immediately seek shelter in the closest refugium while fleeing, which would not accurately reflect a choice based on refugia attributes, and (3) they prefer vertical over horizontal orientation.

Three days before an experiment was started, a group of 25–30 spiders of either species was each offered a German cockroach, *Blattella germanica* L., of appropriate size (as similar to the spider body length as possible). Feeding the *Loxosceles* spiders several days before an assay was considered the best design because they sometimes actively feed up to 24 h (R.S.V., unpublished data). This ensured that spiders were well fed and would have sufficient supply of protein to lay down fresh silk in the new habitat. In addition, spiders that do not eat are typically about to die, are preparing to molt, or are still teneral from a recent molt, and therefore, this eliminated them from testing. We could test a maximum of 20 spiders at one time. Final sample sizes varied from 20 or 40 because of death of a test subject during the experiment; their data were discarded.

Just before transfer, the body length of each spider was estimated to the nearest 0.5 mm with a ruler. Sizes of spiders were chosen to represent as wide a population breadth as possible that were available for testing. The spider was transferred into the test arena, and the arena was covered with a lid. Each arena was immediately moved to the counter top. On the final day of the experiment, the assay was terminated after the morning recording. Each spider was returned to its

original housing vial and lid to not expose them to odors and silks of conspecifics. Refugia were removed and arenas washed in hot water with toweling to remove feces, silk, or other contaminants.

Although observations were recorded for 4 or 7 d in the choice tests, statistical comparisons were performed on data collected on the first observation period. This was the only observation where all spiders were under equal conditions, that is, they were forced to make a choice based on the same variables on the first night. Spiders occupying refugia on the first night laid down silk and possibly chemical cues inside the crevice of the refugium that could influence choices on subsequent nights, complicating the interpretation of the data. Additional analysis on subsequent days was performed when it appeared to add further understanding of the results, but most of the data for day 2 onward, although recorded, were neither analyzed nor presented here.

Size Preference of the Refugia. Because of limited space in the circular arenas, the seven refugia sizes were assayed in groups of four in vertical orientation. The smaller series consisted of the four smallest crevice sizes (3.2, 4.8, 6.4, 9 mm) and the larger series consisted of the four largest crevice sizes (9, 15, 18, 21 mm). The 9-mm refugium was included in both choice tests so that there would be a reference point for comparison. The location of each spider was checked each morning for 4 d. Two replicates of 20 spiders each for each species were performed with the larger-sized refugia and one replicate of 20 spiders for each species tested with the small-sized refugia.

The number of spiders selecting each refugium was analyzed by χ^2 for day 1 (Sokal and Rohlf 1969). The location of each spider on days 2, 3, and 4 was compared with its location on the previous day (χ^2 analysis) to determine whether they moved each night. The association between the body size of each species and their refugia preferences was analyzed by a Kendall coefficient of rank correlation (Sokal and Rohlf 1969).

Orientation Preference. After the previous experiment, two sizes from the middle of the acceptable crevice size range (15 and 18 mm) were chosen for testing the spider's vertical or horizontal orientation preference. Four refugia were offered to each spider in the two crevice sizes and the two orientations. The location of the spiders was checked each morning for 4 d. Two replicates of 20 spiders each for each species were performed.

The data were analyzed using a contingency table and χ^2 tests for day 1 data (Statistix 2005).

Site Fidelity. To determine the degree of site fidelity, 20 spiders were confined in arenas for 30 d. The arenas were set up with four 15-mm crevice refugia in vertical orientation. To determine whether degree of hunger affected the spider's willingness to switch retreats, the experiment consisted of two simultaneously tested cohorts of 10 each: (1) one cohort was fed 3 d before introduction and (2) the other cohort was fed 17 d before introduction to the arenas. A spider was placed in an arena and its location was noted daily for

Table 1. Refugia size preferences of *L. reclusa* and *L. laeta* for refugia on day 1 in choice studies

	No. spiders selecting refugia width (mm) ^a											χ^2	
	Small choice test				χ^2	<i>P</i>	Large choice test				χ^2		
	3.2	4.8	6.4	9			9	15	18	21			
<i>L. laeta</i>	0	1	8	8	9.1	<i>P</i> < 0.05	10	9	12	8	0.9	Not significant	
<i>L. reclusa</i>	1	1	1	17	38.4	<i>P</i> < 0.001	6	12	6	12	3.0	Not significant	

^a The no. of spiders selecting refugia sizes by the following morning. χ^2 tests (Sokal and Rohlf 1969).

the next 30 d, during which spiders were not fed. One replicate of this trial was run with 20 spiders of each species.

The number of times each species changed refugia over 30 d was compared with spiders fed 3 and 17 d before testing by an *R* × *C* test of independence (Sokal and Rohlf 1969). The number of switches for each species was compared with a Kolmogorov-Smirnov test to determine whether there were species differences.

Effect of the Presence of Silk. After terminating the site fidelity assay, 20 refugia for each species, which had visible silk deposited from a spider's occupancy, were saved and kept at room temperature (~25°C) for 8 d. Twenty spiders were offered a choice of a fresh refugium or one with silk of a conspecific. The position of the spiders was checked every morning for 7 d. This assay was performed once for 20 spiders of each species. Data were analyzed using χ^2 for day 1 results for the nonunanimous data.

Results

Size Preference. Neither *Loxosceles* species showed distinct preference for refugia size (Table 1) in the larger refugia choice tests with spaces of 9, 15, 18, and 21 mm. In the smaller refugia choice tests with crevice sizes of 3.2, 4.8, 6.4, and 9 mm, *L. laeta* preferred the two largest crevice sizes and *L. reclusa* preferred the 9-mm refugium. There was no association between body size and the size of the refugia selected by *L. reclusa* (small refugia: $\tau = 0.0064$, *P* = 0.97; large refugia: $\tau = 0.1843$, *P* = 0.27) and *L. laeta* for small refugia ($\tau = 0.0635$, *P* = 0.79). However, larger *L. laeta* preferred larger refugia ($\tau = 0.3187$, *P* = 0.05). The two smallest refugia sizes were only chosen by 3 of the 40 spiders tested of both species on day 1; the limita-

tion seems to be whether or not a spider could squeeze into the crevice. Anecdotally, it seemed if adequate space was available that the spider preferred to position itself inside the refugium on the topmost surface of the vertically oriented refugium, with tarsi touching both vertical sides instead of cramming its body into the crevice in a sideways orientation.

On the second, third, and fourth days, both species preferred refugia that they used the preceding day. Approximately 83, 82, and 91% of *L. laeta* selected the used refugium on days 2, 3, and 4, respectively ($\chi^2 = 103.2$, *P* < 0.001; $\chi^2 = 102.8$, *P* < 0.001; $\chi^2 = 136.3$, *P* < 0.001; respectively). Approximately 96, 93, and 87% of the *L. reclusa* selected the used refugia on days 2, 3, and 4, respectively ($\chi^2 = 152.4$, *P* < 0.001; $\chi^2 = 149.2$, *P* < 0.001; $\chi^2 = 121.7$, *P* < 0.001; respectively).

Orientation Preference. Both *Loxosceles* species preferred refugia with a vertical orientation. Vertical refugia were chosen by 92.3% of the *L. reclusa* spiders ($\chi^2 = 27.9$, *P* < 0.01) and 81% of the *L. laeta* spiders ($\chi^2 = 13.4$, *P* < 0.01) for both the 15- and 18-mm sizes (Fig. 3). In the vertical orientation, neither species showed size preference for 15- or 18-mm crevices (*L. laeta*, $\chi^2 = 1.72$, not significant; *L. reclusa*, $\chi^2 = 0.11$, not significant).

Site Fidelity. The degree of hunger had no influence on the switching behavior of either *L. reclusa* (*G* = 2.39, not significant) or *L. laeta* (*G* = 5.84, not significant). Consequently, all of the distributional data were pooled for each species. Regarding switching, 10 of 19 *L. reclusa* were always found in the same refugium in the morning, 5 switched refugia 2–8 times, and 4 spiders switched 10 or more times (Fig. 4). Five of 20 *L. laeta* spiders were always found in the same refugium in the morning, 8 switched refugia 1–8 times,

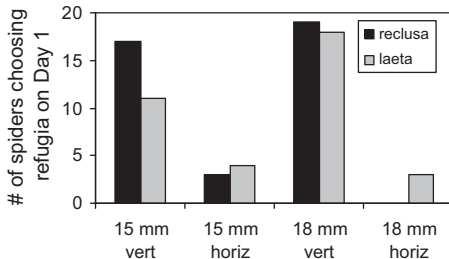


Fig. 3. Orientation preference of *L. reclusa* and *L. laeta* spiders for 15- and 18-mm crevices in vertical and horizontal orientation.

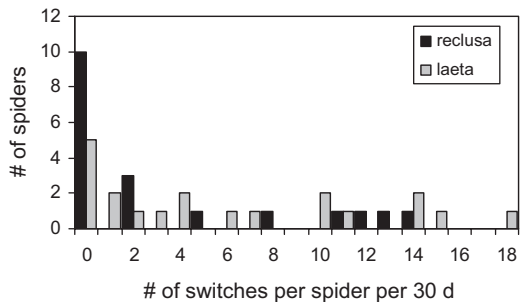


Fig. 4. The number of times individual spiders of *L. reclusa* and *L. laeta* switched refugia over a 30-d period when offered four vertically oriented refugia of 15-mm crevice size.

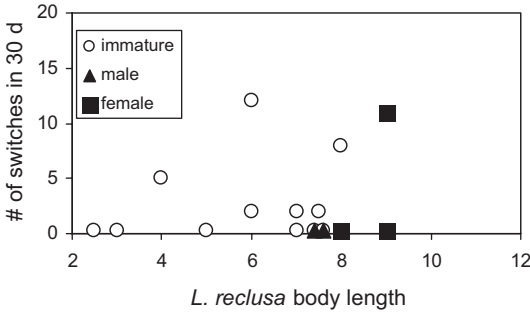


Fig. 5. Body length and lifestage of *L. reclusa* spiders in comparison to the number of switches made between refugia over 30 d.

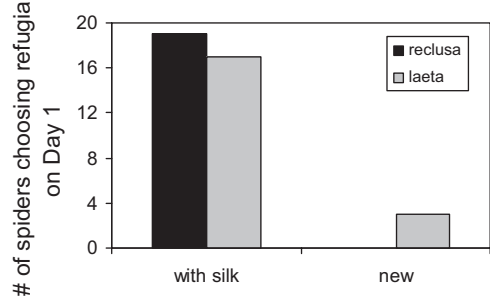


Fig. 7. Preference of *L. reclusa* and *L. laeta* spiders for refugia that were either new or with silk laid down by a previous conspecific occupant during the 30-d trial.

and 7 switched 10 or more times (Fig. 4). There were no significant differences between the two species in their propensity to switch refugia over the 30-d period (mean number of switches: *L. reclusa*, 3.63 ± 5.16 ; *L. laeta*, 6.00 ± 5.96 ; $G_{adj} = 0.28$, not significant). Although there are only 39 data points, there seem to be no obvious trends among the spiders, with switching frequency being as unpredictable for mature or immature spiders of either species (Figs. 5 and 6).

Effect of the Presence of Silk. Refugia used in the site fidelity study that had silk covering a portion of the crevice were significantly preferred over new refugia of the same size by both species (Fig. 7). On day 1, 17 of 20 *L. laeta* selected silked crevices ($\chi^2 = 9.8$, $P < 0.05$) and all 19 *L. reclusa* selected silked crevices.

Discussion

Loxosceles reclusa and *L. laeta* preferred refugia with crevices ≥ 9 and ≥ 6.4 mm, respectively, with no or only slight correlation of body size to crevice width (Table 1). This is in contrast to research where individual German cockroaches preferentially showed a conspicuous affinity for refugia in relation to their body size based on thigmotactic cues (Koehler et al. 1994). This information was used to develop a more efficacious adhesive trap for American cockroaches, *Periplaneta americana* L. (El-Mallakh and Hartmann 2003). However, both *Loxosceles*

species tested here showed definite preferences for a vertically oriented refugium as opposed to horizontal (Fig. 3) and a refugium that had been covered with silk from a conspecific compared with a fresh refugium (Fig. 7). In regard to the silk, it is not known whether this attraction was tactile, pheromonal, or another sensory cue. Additionally, for all tests performed here, we are aware that the *Loxosceles* spiders were forced to make limited choices in a very artificial environment; the attributes presented here may still be suboptimal in a real world situation.

Because *Loxosceles* spiders show site fidelity, this behavior could detrimentally affect the development of a control strategy if spiders are not moving around often, such that they would contact a pesticide-laden refuge. However, in our study, a large percentage of the spiders did indeed switch refugia (Fig. 4). Regarding the others, 53% of *L. reclusa* and 25% of *L. laeta* spiders were always found in the same refugium during the morning observation for 30 consecutive d. There is no indication whether these spiders entered other refugia and returned to the same place or whether they never moved. Levi and Spielman (1964) reported that the removal of a *L. laeta* female from under a board resulted in additional conspecifics moving into the site; each sequential removal resulted in a new resident over a period of about a week. Therefore, there is indeed movement in an infested building scenario. However, in our study, it was hypothesized a priori that mature males might make many switches because of their drive to find mates, whereas, in contrast, the females were assumed to be rather sessile; in reality, each sex was represented by individuals that made no switches and those that switched every 2 or 3 d (Figs. 5 and 6). It was thought that the degree of starvation might influence activity, i.e., sated animals might be more sessile because they did not need to feed. An alternative hypothesis was that sated spiders might be more active because they had plenty of energy reserves. Neither hypothesis proved correct, because degree of starvation had no apparent effect.

If the preferences shown by *Loxosceles* spiders in these experiments in an artificial environment hold true in the real world, the design of a refugium for control of reclusid spiders should incorporate vertically oriented refugia with a width of at least 9 mm.

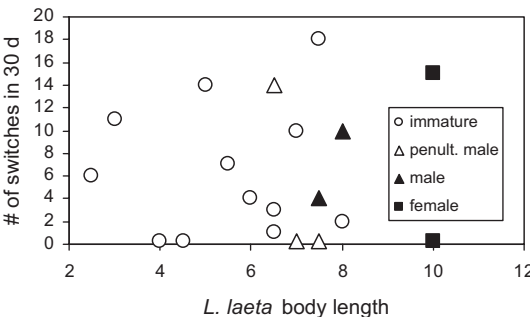


Fig. 6. Body length and lifestage of *L. laeta* spiders in comparison to the number of switches made between refugia over 30 d.

The exact width does not seem to be critical; therefore, this is not a constraint for manufacturing a device for control. There is an attractive component to the silk of conspecifics. However, it is unknown whether the attraction is physical or chemical. If the cue is mechanical, possibly fibrous material could be sprayed on cardboard to enhance attraction. If chemical cues are involved, it may be possible to extract webbing and identify the substance. From a pest management perspective, contaminated stations could offer attractive refugia that recluse spiders might choose for a daytime haven. Such stations could be incorporated into control programs, using devices treated with glue or pesticides to kill or trap spiders. In addition, the non-sex specificity of individuals showing frequent switching indicates that gravid females might be just as susceptible to eradication using this system as males and immatures. Minimal motility of some of the spiders may be less detrimental as initially perceived, e.g., a pesticide-treated station might remove the wandering spiders (those individuals more likely to be involved in envenomations) but not the recluses that show extreme site fidelity and that would be less likely to interact often with humans. Hence, less control efficacy would not be necessarily detrimental. However, site-tenacious gravid females would still be a source of new spiderlings.

Finally, our experiments forced spiders to make refugia choices based on narrow options in a very artificial environment. More research needs to be done to define what is attractive to spiders in a real world situation.

Acknowledgments

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