# ATTEMPTED SUPPRESSION OF A SEMI-ISOLATED *CULEX TARSALIS* POPULATION BY THE RELEASE OF IRRADIATED MALES: A SECOND EXPERIMENT USING MALES FROM A RECENTLY COLONIZED STRAIN

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ABSTRACT. Approximately 85,000 radiosterilized males from a newly established colony of *Culex tarsalis* were marked with fluorescent dust and released in a semi-isolated canyon in the arid Sierra Nevada foothills of Kern County, California, during the spring of 1981. Relative abundance and sterility were monitored in the test canyon and 2 adjacent comparison canyons. Radiosterilized males survived well, dispersed throughout the test canyon and comprised 30% of all males collected. The 11% sterility introduced into the test population after releases commenced was insufficient to suppress or delay the vernal increase in female relative abundance. Overall, radiosterilized males were uncompetitive (29%) against native males for target females. The loss of competitiveness was attributed to the onset of assortative mating related to colonization; i.e., in both outdoor cage and mark-release-recapture experiments, sterile and native males mated more frequently with females of their own genotype.

Studies on the bioregulation of field populations of *Culex tarsalis* Coq. using genetic methods began in Kern County, California, in 1977. Initial field releases utilized a chromosomal interchange system but failed to introduce sufficient sterility into the target population because the released laboratory-adapted semi-sterile males did not mate competitively (Asman et al. 1979, Milby et al. 1980). In a subsequent pilot study significant sterility was introduced into a smaller isolated test population through the release of radiosterilized males reared from pupae collected at a prolific breeding site (Asman et al. 1980). These results led to larger scale releases of irradiated males in 1980 (Reisen et al. 1981). Concomitant outdoor cage and field evaluations indicated the irradiated males mated competitively. However, the release rates remained far below the estimated addition rate to the target population and were insufficient to demonstrate control. These studies indicated that radiosterilization did not impair mating performance and that numerical suppression of the population might be feasible if an adequate sterile male release rate could be achieved.

To provide adequate sterile male production, a colony was established from the target population during late autumn 1980, expanded during the winter and then irradiated and released back into the parent population during early spring 1981, prior to the vernal population increase. Emphasis was placed on the early attainment of a favorable sterile-to-field male ratio without sacrificing male mating competitiveness. The present paper describes this attempted suppression of the spring *Cx. tarsalis* population at the Breckenridge study area in Kern County.

## MATERIALS AND METHODS

STRAINS. *Br80:* Released males originated from a laboratory colony established from more than 3000 females collected by $CO_2$-light trap at Breckenridge during 16–28 September 1980. This colony declined in egg raft production during $F_1$, but by the $F_2$ generation in

November the average fecundity of 42 egg rafts was 197 eggs/raft with 95% hatch. The numbers of egg rafts produced progressively increased with increasing laboratory adaptation. The colony was in the $F_9$ generation at the start of production for release.

*BrW:* Mosquitoes collected as pupae or adults from the Breckenridge population.

*BrW-$F_1$:* The laboratory-reared progeny of field-mated females collected in light traps at Breckenridge, but not selected for mating under laboratory conditions.

*BFS:* The Bakersfield laboratory colony which has been maintained at the Arbovirus Field Station for over 20 years.

REARING, PRODUCTION AND STERILIZATION. *Br80* females were held in $(0.6 m)^3$ cages and were offered a restrained chicken as a blood meal source. A dish filled with tapwater provided an oviposition substrate. Progeny were reared at a density of 5 egg rafts (183 eggs/raft, n = 224 rafts) per rearing tray (surface area = 0.08 m²) in the insectary or 40–60 egg rafts per shower stall bottom (surface area = 0.47 m²) at a mass production facility. Larvae were fed a diet of finely sifted liver powder, Tetramin® and rabbit pellets in a 2:1:2 by-volume ratio. Pupae were culled on alternate days using a mechanical separator (Fay and Morlan 1959). Insectary and production facility conditions were maintained at 25±3°C and 60±20% relative humidity with a 16L:8D photoperiod including simulated dusk and dawn periods of 1 hr each.

During the winter of 1981 the *Br80* colony was expanded to meet projected production needs. Egg rafts produced on days 1, 3, 5, 13, 15 and 17 of a 24-day rotating schedule were used to provide adults for 6 production cages, while egg rafts produced on days 7, 9, 11, 19, 21 and 23 were used to repopulate the *Br80* parent colony. On alternate days pupae produced at the production facility were shipped via bus to the Division of Entomology and Parasitology at the University of California in Berkeley (distance = 450 km), where the males were separated from females within 24 hrs of emergence and counted into 3 equal-sized groups. Males were exposed to 6 KR of gamma radiation from a $Co^{60}$ source at 200 R/min. The following day the radiosterilized males, *Br80(I)*, and most sibling females (unirradiated) were returned to Bakersfield by bus. Females were added to newly established production cages.

RELEASE. The Breckenridge study site consisted of 3 canyons approximately 300 m apart. The 60 m high ridges provided a central test canyon (B) and 2 peripheral comparison canyons (A and C). The *Br80(I)* males were transported to Breckenridge on their afternoon of arrival where they were marked with a site-specific fluorescent dust color (Nelson et al. 1978). Dusted *Br80(I)* males (now 3 days old and sexually mature) were released at least 1 hr before sunset, in equal numbers, at 3 release sites situated near light trap locations 1, 2 and 4 in Canyon B (see Fig. 1 of Reisen et al. 1981 for light trap positions). Males not dispersing from the transport cartons were considered dead and subtracted from the number irradiated to estimate the actual number released.

ASSESSMENT. Mosquitoes were collected at least twice weekly from 22 March through 14 July 1981 using the following methods:

Twelve CDC miniature light traps augmented with 1–2 kg dry ice were operated at the same stations used previously (Reisen et al. 1981). An additional 4 traps were positioned within 50 m of the central release site (at trap 2) and operated without light bulbs to collect additional females for sterility monitoring.

Three walk-in and 12 standard red box shelters were positioned near trap 2 and were sampled in late afternoon and early morning.

Swarming males were collected at dusk at 6 fixed sites located within 50 m of trap 2. Supplementary collections were made when additional swarms could be located. Sampling effort was comparable at each swarm, with males collected by sweeping with an aerial net for 3–5 min.

A truck trap (Nelson and Bellamy 1971) was operated in Canyon B for 50 min starting 10 min before swarming commenced. Ten runs of 2 min duration were made at 5 min intervals over a 0.86 km course starting near trap 1, proceeding west around trap 4, then returning past trap 3 to trap 1 (see Fig. 1 of Reisen et al. 1981). The truck trap route passed all 3 $Br80(I)$ male release sites.

Mosquitoes from all collections were returned to the laboratory, anaesthetized with chloroform, examined for fluorescent dust under ultraviolet light and sorted by species and sex. $BrW$ females collected by CDC traps from each canyon were offered a restrained chick as a blood meal source. Blood-engorged or gravid females were isolated in vials for oviposition and the resulting egg rafts differentially counted as described previously (Reisen et al. 1981). The first 20 egg rafts per sampling date and then every 4th or low hatch raft were counted. The remaining rafts with greater than 90% hatch were scored as "high hatch" after microscopic examination and were not actually counted. All females that oviposited completely unembryonated egg rafts were dissected to determine their insemination status. In addition, egg rafts were collected sporadically from natural breeding sites in each canyon, held individually for hatching and then counted as above.

The absolute size of the $BrW$ male population in Canyon B was estimated on each sampling occasion using the Yasuno and Rajagopalan (1977) modification of the Lincoln index. These estimates presumed that the ratio of recaptured $Br80(I)$ males to all $Br80(I)$ males remaining in Canyon B was equal to the proportion of the unmarked $BrW$ male population sampled. The addition rate to the $BrW$ male population was then calculated by the method of Manly and Parr (1968).

QUALITY CONTROLS. Production efficiency was monitored by recording the numbers of egg rafts used to provide the adults for the 6 production cages and the release cohorts. Egg raft size and fertility were monitored from samples of rafts isolated in vials for hatching. The numbers of $Br80$ adults released into each production cage were estimated by the strip count method of Dow et al. (1965). The numbers of $Br80$ pupae shipped to Berkeley were estimated on several occasions by counting the number of pupae in 1 cc and multiplying by total volume to estimate cohort size (Muhktar et al. 1980). The numbers of males to be irradiated were hand-counted during sex separation.

The sterility of the released $Br80(I)$ males was verified by crosses to $Br80$ or $BFS$ females in laboratory cages. Blood fed females were processed as described above.

The ability of the laboratory colonized and acclimatized $Br80(I)$ males to survive and mate under semi-natural field conditions was determined monthly by holding cohorts of 50 dusted $Br80(I)$ males with 50 $Br80$ females in $(0.6 m)^3$ cages at Breckenridge. Control cages of 50 pairs of $BrW$ adults emerging from field-collected pupae were established concurrently. Adults were continually offered 10% sucrose and the cage tops were covered with wetted disposable diapers to increase humidity. After the 4–6 day test period, adults were removed, counted and the females dissected to determine insemination status and ovarian condition. Since the females were never offered a blood meal source, individuals with follicles developed beyond stage II were considered autogenous. The survivorship of uncaged $Br80(I)$ and $BrW$ males marked with a cohort-specific dust color was estimated at monthly intervals from the exponential decline in recaptures during a 10-day period (Gillies 1961).

COMPETITIVENESS. The mating competitiveness of the $Br80(I)$ males against $BrW$ males for $Br80, BrW$ and $BrW\text{-}F_1$ females was assessed in outdoor quonset hut cages and at Breckenridge using the method of Haisch (1970). The procedures of Terwedow et al. (1977) were modified for assessment in quonset hut

cages. Adults of all genotypes were held on a natural photoperiod in the insectary until sexually mature (4–7 days old), released into the cages at dusk and then recaptured the morning after the 3rd night of cohabitation. At Breckenridge fluorescent-dusted females were recaptured for 10 consecutive days starting the night of release. All females were separated from males on the day of collection and processed as described above.

## RESULTS

PRODUCTION AND RELEASE. The projected production efficiency, based on productivity of the *Br80* colony during $F_2$ in Nov 1980, was 41 males/egg raft. A total of 2,700 egg rafts oviposited by the *Br80* parent colony produced 23,336 males and 15,969 females to populate the 6 production cages. Starting in April, these cages were augmented with females from previous production lines. Overall, the 6 production cages yielded 14,815 egg rafts which were used to establish 43 release lines in shower stall bottoms. Rearing success to pupation in shower stall bottoms averaged 3,600 pupae/stall or 72 pupae/raft. However, considerable mortality occurred during pupation. On 3 occasions, 133 cc of pupae were culled at Bakersfield and shipped to Berkeley for irradiation. With 1 cc = 277 pupae, a projected 59% males and an emergence success of 86%, the 133 cc should have resulted in 18,693 *Br80(I)* males; however, only 6,689 males were actually produced. The cause for this 64% pupal loss is undetermined. To date, 13 rearing experiments to evaluate water source, temperature, food quality and quantity, food particle size, aeration, density and the use of selected antibiotics have failed to determine the cause of the poor immature survival.

Actual productivity during mass production averaged 9.3 males/raft in standard rearing trays and 6.3 males/raft in shower stall bottoms. A total of 87,672 *Br80* males were irradiated and shipped from Berkeley, of which 84,652 (96.5%) were released at Breckenridge on 39 occasions from 28 Mar through 14 Jun 1981 ($\bar{x} = 2,171$ males/release, max = 3,992 on 14 May, min = 423 on 28 Apr, Fig. 1a).

MALE POPULATION DYNAMICS. Of the 84,652 *Br80(I)* males released, 1,287 (1.5%) were recaptured, all in Canyon B. Since recapture efforts which favored the sampling of males (swarm and red box collections) were concentrated near trap 2, significantly more males released at site 2 were recaptured (2.8%) than were males released at sites 1 (0.86%) or 4 (0.78%) (P < 0.05). Only the truck trap which passed near all release sites recaptured comparable proportions of *Br80(I)* males released at each site. *Br80(I)* males mingled well with the *BrW* population in Canyon B, although a slight, but significant, heterogeneity was observed among the proportions of *Br80(I)* males among all males collected by each method: CDC light trap = 31% (n = 504), red box = 27% (n = 1,470), swarms = 31% (n = 2,026, truck trap = 35% (n = 331), $\chi^2_3$ = 10.8, P < 0.05. The overall proportion of *Br80(I)* males among all males (0.30) was significantly less than 0.5 or a 1:1 ratio (P < 0.05). The proportion of *Br80(I)* males varied temporally with the numbers released (Fig. 1a) and with the vernal increase of the *BrW* population (Fig. 1b), but did not significantly differ from 0.5 during weeks 2, 3, 4 and 8 of release (Fig. 1c).

The daily survivorship of the *Br80(I)*

Fig. 1. Weekly estimates of (a) numbers of *Br80(I)* males released per week and remaining in Canyon B on the last day of the week; (b) *BrW* male mean daily population size in Canyon B, total additions per week (7X mean daily additions) and relative abundance (total *BrW* males collected per sampling occasion); and (c) percent of *Br80(I)* males among all males (encircled points were significantly less than a 1:1 ratio, P < 0.05) and sterile rafts sired by *Br80(I)* males among all rafts from Canyon B; Breckenridge, Kern County, California, 1981.
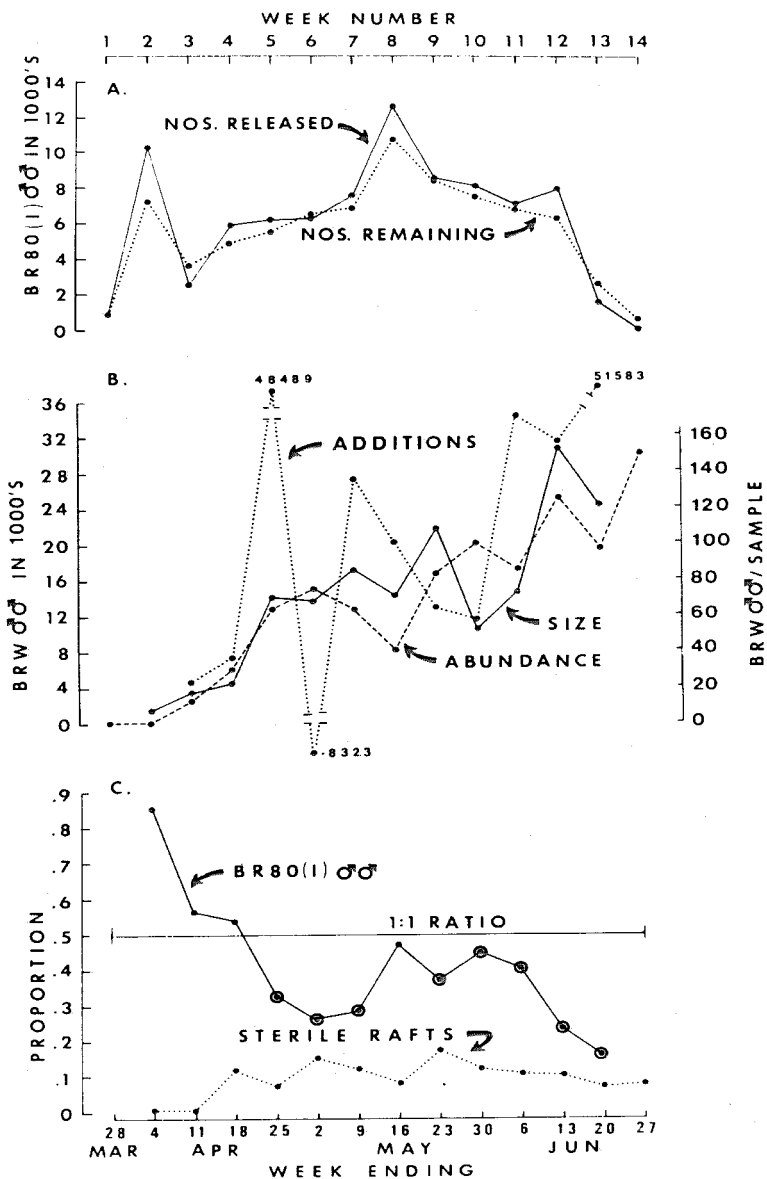
males dusted with both site- and date-specific colors equaled 0.86 in Apr and 0.83 in Jun. Unstable weather during the May release resulted in a variable recapture rate and the calculated regression coefficient did not differ significantly from 0 ($P > 0.05$). A pooled survivorship estimate of 0.84 was used to calculate the numbers of $Br80(I)$ males remaining in Canyon B on each day of the sterile male release period ($\bar{x} = 5,711$, max = 10,833 on 16 May, Fig. 1a). Weekly $BrW$ male population size and addition rate were averaged from daily estimates calculated for each sampling occasion (Fig. 1b). The vernal increase in estimated male population size was well correlated temporally with male relative abundance ($BrW$ males collected by all methods per sampling occasion) in Canyon B (Fig. 1b, $r = 0.86$, $P < 0.01$). From 5 Apr through 20 Jun, the estimated $BrW$ male addition rate averaged 22,055 males/week (7X daily mean) and was significantly higher than the mean $Br80(I)$ male release rate of 6,772 males/week during the same period (Fig. 1a,b, paired $t_9 = 2.76$, $P < 0.05$).

QUALITY CONTROLS. *Sterility of $Br80(I)$ males.* Egg rafts from $BFS$ or $Br80$ females mated to $Br80(I)$ males sampled from the release cohorts exhibited characteristic low hatch and embryonation patterns: hatch = 3.5%, range = 0 to 28%; embryonation = 9.8%, range = 0 to 47%; n

= 169 egg rafts. Some females with sperm visible in their spermathecae oviposited completely unembryonated egg rafts. Hence, rafts with all unembryonated eggs oviposited by inseminated females were attributed to $Br80(I)$ male matings. Egg rafts from $BrW$ females mated with $Br80(I)$ males in a quonset hut cage exhibited similar hatch and embryonation patterns: hatch = 3.6%, embryonation = 9.6%, n = 147. These rafts were readily distinguishable from those from $BrW$ females mated with $BrW$ males in a quonset hut cage: hatch = 75%, embryonation = 76%, n = 88. In assessing rafts from field-collected females, rafts with characteristic hatch (< 30%) and embryonation (< 40%) patterns were attributed to $Br80(I)$ male matings.

*Fitness of $Br80(I)$ males.* The $Br80(I)$ males were able to survive and mate in cages at Breckenridge during early spring (Table 1). The proportions of $Br80(I)$ and $BrW$ males surviving the test period did not differ significantly except in April. The proportion of $Br80$ females inseminated by sibling $Br80(I)$ males was significantly greater than the proportion of $BrW$ females inseminated by $BrW$ males. Reduced mating by $BrW$ adults was attributed to their lack of selection for mating while confined in cages. During April when temperature averaged 18°C, the proportion of $Br80$ females inseminated

Table 1. Survivorship and mating performance of laboratory-adapted and acclimatized $Br80(I)$ males compared to $BrW$ males from the target population while held in cages at Breckenridge.[1]

| Date (temp.) | Genotypes | | Days of test (T) | No. alive | | Survivorship[2] | | Percent | |
|---|---|---|---|---|---|---|---|---|---|
| | ♂ | ♀ | | ♂ | ♀ | ♂ | ♀ | Insem. | Autog. |
| 10 Apr | $Br80(I)$ | $Br80$[3] | 6 | 32[a] | 42[a] | .93 | .97 | 57[a] | 21[a] |
| (18° C) | $Br80(I)$ | $Br80$ | | 38[a] | 49[a] | .96 | 1.0 | 41[a] | 8[b] |
| | $BrW$ | $BrW$ | | 47[b] | 50[a] | .99 | 1.0 | 4[b] | 6[b] |
| 20 May | $Br80(I)$ | $Br80$ | 6 | 13[a] | 24[a] | .80 | .88 | 79[a] | 8[a] |
| (22° C) | $BrW$ | $BrW$ | | 7[a] | 16[a] | .72 | .83 | 19[b] | 6[a] |
| 14 Jun | $Br80(I)$ | $Br80$ | 4 | 20[a] | 34[a] | .80 | .91 | 77[a] | 12[b] |
| (27° C) | $BrW$ | $BrW$ | | 22[a] | 44[b] | .81 | .97 | 14[b] | 34[b] |

[1] 50 pairs/cage; proportions or numbers within monthly trials followed by the same letter were not significantly different when tested by $\chi^2$ ($P > 0.05$).

[2] Daily survivorship = (no. alive/50)$^{1/T}$.

[3] Comparison cage held in insectary.

in the field and the laboratory were not significantly different (P < 0.05) indicating that low temperature did not inhibit the *Br80(I)* males from mating. The autogeny rate (i.e., the proportion of females developing follicles beyond stage IIb without imbibing a blood meal) of the *Br80* and *BrW* females was included as an index of cohort quality at emergence. Autogeny in *Cx. tarsalis* is best expressed in females reared under uncrowded conditions with sufficient food quality and quantity. The proportion of autogenous *Br80* females was comparable to that of *BrW* females during April and May, but was significantly less during June (P < 0.05, Table 1). In addition, regression estimates of daily survivorship for *Br80(I)* and *BrW* males dusted and released concurrently at site 2 during June were 0.83 and 0.82, respectively. Survivorship and insemination rates indicated that colonization, mass production, irradiation and dusting did not alter *Br80(I)* survival and ability to mate under field climatic conditions.

*Mating competitiveness.* *Br80(I)* males were statistically uncompetitive (e < 1, P < 0.05) against *BrW* males for *BrW* females in both quonset hut and field tests (Table 2 a,d,e,f). Weekly competitiveness estimates using the sterility monitoring data from 18 April to 20 June (Fig. 1c) were all significantly less than 1 and ranged from 0.11 on 16 May to 0.52 on 13 Jun. *Br80(I)* evaluated during the June mark-release were significantly more competitive than *Br80(I)* males throughout the release period (Table 2 e,f vs. d). Results in quonset hut cage tests agreed with results estimated concurrently at Breckenridge (Table 2 a vs. f, $\chi^2$ = 1.86, P > 0.05). However, when mating competitiveness was evaluated using *Br80* females, the *Br80(I)* males were supercompetitive against the *BrW* males (e > 1, Table 2 c,h). Competitiveness estimates obtained in the quonset hut cages and in nature at Breckenridge were comparable (Table 2 c vs. h, $\chi^2$ = 1.76, P > 0.05), but were significantly greater than competitiveness estimates using the *BrW* females (Table 2 c vs. a and h vs. e,f, P < 0.05). Interestingly, competitiveness estimations using *BrW-F_1* females which were reared under laboratory conditions, but not

Table 2. Competitiveness of *Br80(I)* males against *BrW* males for 3 genotypes of females in quonset hut cages and in the field at Breckenridge, 1981.

| Exp. no. | Female genotype[1] | Month | Percent sterile (n) | | Comp.[4] | $\chi^2_1$[5] |
|---|---|---|---|---|---|---|
| | | | Rafts[2] | Males[3] | | |
| | | Quonsets | | | | |
| a | *BrW* | Jun | 21(29) | 50(1600) | 0.26[a] | 9.97** |
| b | *BrW-F_1* | Jul | 57(31) | 50(1600) | 1.33[ab] | 0.43[ns] |
| c | *Br80* | Jun | 68(34) | 50(1600) | 2.09[b] | 4.24* |
| | | Breckenridge | | | | |
| d | *BrW*-um | Apr–Jun | 11(1219) | 30(4331) | 0.29[c] | 209.71*** |
| e | *BrW*-um | Jun | 8(349) | 13(862) | 0.56[a] | 7.75** |
| f | *BrW*-dusted | Jun | 12(101) | 19(256) | 0.58[a] | 3.17[ns] |
| g | *BrW-F_1* | Jun | 19(31) | 19(256) | 1.04[a] | 0.00[ns] |
| h | *Br80* | Jun | 45(196) | 19(256) | 3.53[b] | 86.67*** |

[1] Genotype designations follow methods section; um = unmarked field adults collected from the Breckenridge Canyon B population.
[2] Percent of egg rafts attributed to matings by *Br80(I)* males.
[3] Percent of *Br80(I)* males among all males during days when mating was assumed to have occurred.
[4] Competitiveness; values followed by the same letter were not significantly different when tested as described by Grover et al. (1976).
[5] Value of $\chi^2$ for testing the null hypothesis that competitiveness = 1; *** = P < 0.001, ** = P < 0.01, * = P < 0.05, ns = P > 0.05.

selected for mating in cages, were approximately equal to 1 and thus were intermediate between values obtained using *BrW* and *Br80* females. These results indicate non-genetic factors may also be involved. Again, comparable results were observed in the quonset hut cage and at Breckenridge (Table 2 b vs. g, $\chi^2$ = 0.14, P > 0.05).

SWARMING BEHAVIOR. Male swarms were collected at 6 fixed stations in the site 2 area from 12 Apr through 20 Jun. Three "top" swarms formed over *Atriplex* bushes and young *Tamarisk* trees, while 3 "space" swarms formed within breaks in a row of young *Tamarisk* trees growing along a watercourse. A total of 816 and 499 unmarked *BrW* males and 51 and 462 *Br80(I)* males were collected at top and space swarms, respectively. The proportion of *Br80(I)* males collected at top swarms (9.9%) was significantly less than that of unmarked *BrW* males (62%) ($\chi^2$ = 402, P < 0.001). *Br80(I)* males released at sites 1 and 4 accounted for 34% of the 513 *Br80(I)* males collected and their distribution between top and space swarms did not differ significantly from that of *Br80(I)* males released at site 2. This indicated that the reduced number of *Br80(I)* males collected within top swarms was not necessarily related to the juxtaposition of the release and swarm sites. In addition, significantly fewer *Br80(I)* males were found in top swarms than marked *BrW* males which were collected as pupae at Breckenridge, allowed to emerge in the laboratory and released at site 2 when 1–2 days old (29%, n = 120). Marking and release also may have had some effect on swarming behavior, since proportionately fewer dusted than unmarked *BrW* males were taken at top swarms.

TARGET POPULATION STERILITY MONITORING. Before the initiation of *Br80(I)* male releases, 46 egg rafts collected in Canyon C exhibited high hatch. During the release period, 11% of 1,218 egg rafts which were oviposited by *BrW* females collected in Canyon B were sterile and considered to be sired by *Br80(I)* males. The proportion of sterile rafts in Canyon B was significantly higher than that observed in comparison Canyons A (2.2% of 457) or C (7.2% of 432) (P < 0.05). Comparable sterility was detected among field-collected egg rafts from Canyons A (1.7% of 178) and B (11.6% of 215); however, *Br80(I)* male matings were not detected in 39 egg rafts from Canyon C. The incidence of sterile rafts from unmarked *BrW* females collected after the *Br80(I)* male release period decreased significantly (P < 0.05) in Canyons B (1.9% of 481) and C (1.4% of 139), but not in Canyon A (2.0% of 51).

Despite the introduction of 11% sterility into the Canyon B population, no noticeable decrease in female relative abundance (nos./light trap night) was observed during the sterile male release period (Fig. 2). In fact, the relative abundance of the test Canyon B population was higher than comparison Canyons A and C at the end of the release period. In addition, no delay was detected in the expected vernal population increase.

## DISCUSSION

Male descendants from Breckenridge females collected during autumn were uncompetitive for Breckenridge females against Breckenridge males when released the following spring, after being colonized for 9–16 generations (ca. 6–9 months). Radiosterilization was not considered detrimental, since radiosterilized males emerging from field-collected pupae were fully competitive under similar field conditions at Breckenridge in 1980 (Table 3) and in previous quonset hut experiments (Ainsley et al. 1980, Zalom et al. 1981). The loss of competitiveness was also not attributed to loss of "fitness," since the survivorship of the *Br80(I)* males estimated in cages and by mark-release-recapture methods was the same as that of the males from the target population and *PWW(I)* males released in 1980 (Table 3). In addition, *Br80(I)* males were super-competitive for *Br80* females. The loss of competitiveness was attributed to the onset of assortative mating
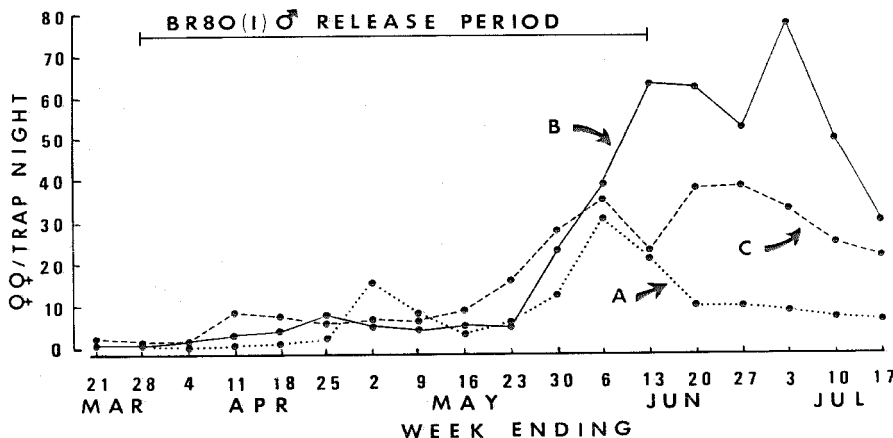
Fig. 2. Weekly average of *BrW* females collected per trap night in CDC light traps augmented with dry ice in 3 canyons at Breckenridge, Kern County, California, 1981.

associated with colonization; i.e., *BrW* and *Br80* females mated more frequently with males of their own genotype. Similar results were obtained with *Cx. tritaeniorhynchus* Giles carrying a chromosomal interchange system, where assortative mating evolved within 3 generations of laboratory maintenance (Reisen et al. 1980). That rapid selection may also occur with *Cx. tarsalis* was suggested by the intermediate results obtained when competitiveness was estimated using the *BrW-F₁*

Table 3. Summary of the 1980 and 1981 releases of radiosterilized male
*Culex tarsalis* at Breckenridge.[1]

| Male genotype[3] | 1980[2] PWW(I) | 1981 Br80(I) |
|---|---|---|
| Generations in insectary | 0 | 9–16 |
| Dates released | 17 Jun–28 Aug | 28 Mar–14 Jun |
| Total males released | 71,016 | 84,652 |
| Number of releases | 38 | 39 |
| Release sites in Cyn B | 2 | 1,2,4 |
| Sterile male survivorship | 0.82 | 0.84 |
| Max. sterile males in Cyn B | 10,255 | 10,833 |
| Average percent sterile males | 6.5[a] | 29.7[b] |
| Average percent sterile rafts | | |
|   Cyn A | 8.3[a] | 2.2[b] |
|   Cyn B | 10.0[a] | 11.0[a] |
|   Cyn C | 8.3[a] | 7.4[a] |
| Average sterile male competitiveness in Cyn B | 1.10[a] | 0.29[b] |

[1] Values in the same row followed by the same letter were not significantly different (P < 0.05).
[2] Data from Reisen et al. 1981.
[3] *PWW* = males emerging from field-collected pupae from Poso West; *Br80* = males produced under insectary conditions from a colony originated from females collected at Breckenridge; *(I)* = both types of males exposed to 6000 R at 200 R/min from Co⁶⁰.

females. Presumably a similar loss of mating competitiveness and onset of assortative mating contributed to the failure of earlier control attempts with *Cx. tarsalis* males carrying chromosomal interchanges (Asman et al. 1979, Milby et al. 1980).

The nature of changes relating to colonization remains poorly understood. In culicine mosquitoes the ability to mate under laboratory conditions is generally considered to be a female mediated event and it is female behavior which presumably undergoes the greatest modification during colonization (Sasa et al. 1967, O'Meara and Evans 1974, McDonald et al. 1979). However, changes must occur in the male as well, since in nature field-type females mate less frequently with laboratory-adapted males. A possible contributing factor which could have resulted in a reduction in the number of *Br80(I)* male contacts with *BrW* females was the low number of *Br80(I)* males collected at top swarms. If the opportunity for mating was related to male abundance in swarms, then proportionately fewer *Br80(I)* males may have been swarming in the correct place. The actual importance of heterogeneous swarming patterns to the relative contribution of males to the population gene pool remains unresolved and will be the subject of further research.

Despite the poor mating competitiveness of the *Br80(I)* males, 11% of the rafts from *BrW* females and 12% of egg rafts collected from test Canyon B were classified as "sterile." That the observed low hatch rafts were attributable to *Br80(I)* matings was indicated by characteristic hatch and embryonation patterns and by the decrease in sterility to 1.9% after the termination of releases. The background sterility of the Breckenridge population was estimated to be 2.0% in 1979 (Asman et al. 1980) and 2.9% in 1980 (Reisen et al. 1981). During 1980 and 1981 comparable numbers of radiosterilized males were released and comparable proportions of low hatch rafts were oviposited by females collected

from Canyon B (Table 3). During 1981 lower mating competitiveness was compensated for by the higher proportion of radiosterilized males among all males. Thus, although our release rate was lower than desired, we did enhance the proportion of sterile males significantly by releasing the sterile males during early spring, prior to the vernal population increase.

Results of the present study suggested that our release strategy and *projected* release rate were suitable to suppress the vernal spring increase in the Breckenridge population. Although mating competitiveness was reduced as a result of colonization and/or production, our results suggested that this reduction could be compensated by a greater sterile-male to target-male ratio. Other sterile male programs using irradiation have adopted this approach and demonstrated that relative success could be achieved with an increased release rate (Patterson at al. 1975, 1977). Future research will emphasize production and handling procedures to improve the quality and quantity of the sterile males for release.

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