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ATTEMPTED SUPPRESSION OF A SEMI-ISOLATED POPULATION OF *CULEX TARSALIS* BY RELEASE OF IRRADIATED MALES

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ABSTRACT. During the summer of 1980, an attempt was made to numerically suppress a semi-isolated population of *Culex tarsalis* by releasing radiosterilized males. A total of 71,016 males was collected as pupae from a productive source, Poso West, sterilized by exposure to 6 KR of gamma radiation within 24 hr of emergence and released at Breckenridge, 12.5 km east of Bakersfield in Kern County, California. The incidence of sterility in egg rafts oviposited by females collected in CO₂-

augmented light traps increased significantly from 2.9% prior to sterile male releases to 9.2% during the release period. The mating competitiveness of the sterile males was estimated to be 1.1 based on the proportions of sterile males among all males and sterile egg rafts among all egg rafts. Even though the radiosterilized males mated competitively, the numbers released were insufficient to numerically suppress the target population.

Feasibility trials towards the genetic control of *Culex tarsalis* Coquillett were initiated in 1977 and 1978 at a semi-isolated site in Kern County, California by the release of males carrying a sex-linked double heterozygous translocation (Asman et al. 1979, Milby et al. 1980). The target population was not suppressed numerically which was attributed, in part, to the low mating competitiveness of the laboratory-adapted genetically altered

males (Milby et al. 1980). Release of *Cx. tritaeniorhynchus* Giles males that carried a similar genetic control system yielded comparable results in Pakistan (Baker et al. 1979). These studies suggested that the colonization of *Culex* mosquitoes may rapidly alter mating behavior, resulting in a competitive disadvantage for mating when such males are released back into a field population (Reisen et al. 1980).

In a subsequent pilot study, 13,500 *Cx.*

tarsalis males emerging from field-collected pupae were radiosterilized and released at a semi-isolated site in Kern County during the summer of 1979 (Asman et al. 1980). The incidence of sterile eggs oviposited by wild-caught females from the target population increased significantly from 2% ($n = 50$) before release to 18% ($n = 120$) after release. These observations suggested that the changes in mating behavior related to colonization were circumvented and were sufficiently encouraging to warrant a larger scale population suppression attempt the following year. The use of sterile males emerging from wild-caught pupae was believed to be advantageous because: 1) high sterility could be induced by irradiation with little loss of mating competitiveness (Ainsley et al. 1980); 2) large numbers of pupae would be available from nearby Poso West (Nelson et al. 1978) precluding the need for colonization and mass production; and 3) released males presumably would retain their natural mating behavior as they had not undergone laboratory colonization. The present paper describes an attempt to suppress a *Cx. tarsalis* population using irradiated males collected as pupae.

METHODS AND MATERIALS

DESCRIPTION OF THE STUDY AREA. The Breckenridge study site (Lat: $35^{\circ}23'$, Long: $118^{\circ}49'$) situated in the arid Sierra foothills of Kern County, California, consists of 3 relatively parallel canyons (A, B and C) separated by ridges ca. 60 m high (Fig. 1). Each canyon contains a series of shallow evaporation-percolation ponds that are used for disposal of waste-water from nearby oil fields. The hillsides are irrigated by sprinkling.

During 1980, mosquito breeding was restricted to water-courses connecting the ponds, and to puddles and hoof-prints filled by the sprinkler system. At the onset of the study, the most productive breeding site in all canyons was a small pool below one pond in Canyon B, and all ir-

radiated males were released at this site (R in Figure 1).

Mean weekly maximum temperatures ranged from 25° to 42° C and minimum temperatures from 13° to 27° C during the course of the study. There was only 6.1 mm of rain from May through October, 1980. Thus, the surrounding hillsides outside the irrigated study area were dry and devoid of mosquito breeding, which isolated the target population.

STRAINS. The strains of *Cx. tarsalis* used for study were: 1) *PWW* = wild-caught pupae collected from water-courses at Poso West, located about 18 km north-

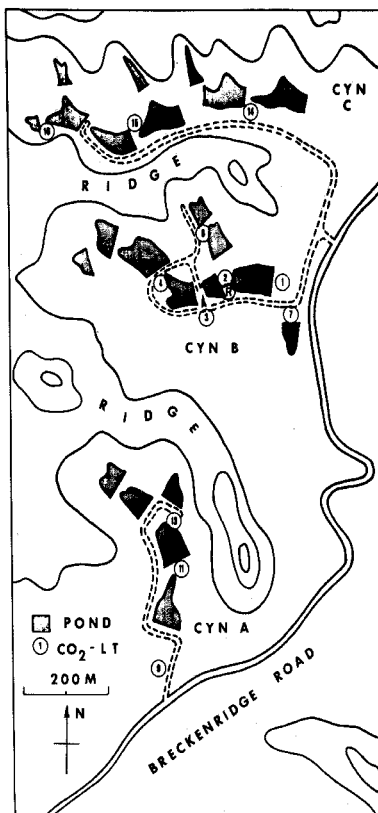


Fig. 1. Breckenridge study site, Kern County, California; R = release site.

west of Breckenridge; 2) *PWW(I)* = radiosterilized *PWW* adults; 3) *BrW* = wild-caught pupae or adults from Breckenridge; 4) *PWC-79* = a laboratory colony from Poso West established during the summer of 1979.

COLLECTION, STERILIZATION, MARKING AND RELEASE. *PWW* pupae and associated 3rd and 4th instar larvae were collected on alternate days from June 10 through August 23, 1980. Pupae were "picked" using a mechanical separator (Fay and Morlan 1959) and held at 5°C to slow development. Larvae were reared outdoors on a 1:4:4 by weight mixture of finely ground brewer's yeast, Tetramin¹ and rabbit pellets.² Reared pupae were picked and combined with subsequent field collections. Pupae were accumulated for 2 days at Bakersfield and then, shipped via bus to the Division of Entomology and Parasitology at the University of California, Berkeley (distance = 450 km). Males were counted and separated from females within 24 hr of emergence. Males were sterilized by exposure to 6000 R of gamma radiation from a Co⁶⁰ source at 200 R/min. Sterile males, *PWW(I)*, were divided equally among 3 containers, offered 10% sucrose on cotton pledgets, returned by bus to Bakersfield and held overnight in an incubator at 26°C. The following morning, the now 3-day-old and sexually mature *PWW(I)* males were transported to Breckenridge where the mosquitoes in one container (i.e., 1/3 of total) were marked with a day- or week-specific fluorescent dust color using the procedures of Nelson et al. (1978). For release, containers were placed upright on the ground, the gauze tops removed and the cartons agitated to induce mosquito dispersal. Mosquitoes that did not leave the cartons were considered dead and subtracted from the total irradiated to calculate the number released. All releases were completed prior to sunrise,

thus enabling the *PWW(I)* males to disperse to suitable microhabitats and intermingle with the target population.

POPULATION MONITORING AT BRECKENRIDGE. *PWW(I)* males and *BrW* males and females were sampled as follows: 1. Twelve CDC miniature light traps (Sudia and Chamberlain 1962), augmented with 1-3 kg of dry ice, were operated from dusk to dawn at fixed sites for at least one night a week from March 30 through October 26, 1980. Trap deployment among the 3 canyons is shown in Fig. 1.

2. Two walk-in (1.8 × 1.2 × 1.2 m) and 11 standard (30 × 30 × 30 cm) red box shelters (Goodwin 1942) were positioned under and around Tamarisk trees within 50 m of the release site. Resting adults were collected from shelters at dawn in conjunction with light trap collections.

3. Adults flying over bushes near light trap sites 2 and 7 in Canyon B were collected by sweeping with an aerial insect net for ca 30 min starting at sunset. Collections were performed sporadically in conjunction with monthly mark-release-recapture studies.

Mosquitoes from all collections were returned to the laboratory, anesthetized with chloroform, examined for the presence of fluorescent dust under an ultraviolet lamp and sorted by species and sex.

MARK-RELEASE-RECAPTURE STUDIES. Horizontal daily survivorship, absolute population size and loss and addition rates for the target population were estimated monthly from May through September using mark-release-recapture methods. Pupae collected at Breckenridge were accumulated for 2 days and allowed to emerge into 3.8 liter carton cages. The numbers of males and females in each cage were estimated by replicate strip counts (Dow et al. 1965), transported to Breckenridge, marked with a date- and genotype-specific dust color and released at dawn (R in Figure 1). Recaptures of these *BrW* adults were attempted for the following 10 days using the collection and processing methods described in the population monitoring section.

¹ Tetra SM® tropical fish food, Tetra, Inc., West Germany.

² Rabbit Family® pellets, Carnation Co., U.S.A.

STERILITY MONITORING. *BrW* females collected by CO₂-light traps in each canyon were offered a chicken as a blood-meal source, held for a minimum of 3 days and then isolated individually for oviposition. The resulting rafts were allowed 3 days to hatch and then counted differentially to determine fertility. Up to 150 *BrW* females from Canyon B and 75 *BrW* females from Canyons A and C were evaluated weekly, although sample sizes were contingent upon the success of light trap catches and female survival.

ANALYSIS OF MONITORING DATA. Survivorship in Canyon B was estimated from the number of marked adults recaptured each day by the regression method (Nelson et al. 1978). The loss rate (death + emigration) was 1 minus survivorship. The number of irradiated males remaining in Canyon B was estimated from the survivorship of released *PWW(I)* males (Milby et al. 1980). Mating competitiveness was estimated by the method of Grover et al. (1976) and tested by Chi-square for significant departure from equal competitiveness (Reisen et al. 1980).

CONTROLS. Concurrent with production and release 5 control studies were performed:

1. Up to 50 egg rafts per wk were collected from Poso West and examined to establish the fertility pattern in an untreated population. Rafts were isolated in vials, held for 3 days and the number of nonembryonated, unhatched embryonated and hatched eggs per raft was counted. Although fecundity declined somewhat in midsummer, the proportion of high hatch rafts (>70% hatch) remained high throughout (98%, $n = 902$ rafts), and sterility was considered a rare occurrence in this population.

2. Prior to the release of *PWW(I)* males, females were collected in CO₂-light traps at Breckenridge to determine the background sterility in the target population. Females were allowed to feed on a chicken and then isolated individually in vials for oviposition. The resulting egg rafts were held for at least 3 days to en-

sure hatching and then counted differentially. Only 3 of 103 rafts (2.9%) exhibited low hatch (<15% hatch, <30% embryonation). This "background sterility" was expected to occur in similarly handled *BrW* females. A similar incidence (2% of 50 rafts) was reported by Asman et al. (1980).

3. The degree of sterility induced by confining ovipositing females in vials was determined for groups of females collected in CO₂-light traps at Breckenridge and Poso West. *BrW* females were blood-fed on a chicken and divided into 2 groups. Group 1 was isolated in oviposition vials, while group 2 was confined in a 3.8 liter cage and allowed to oviposit in a dish. Resulting egg rafts were counted differentially. None of the 44 rafts laid in the egg dish and 5 of the 64 rafts laid by females isolated in vials exhibited low hatch ($P > 0.05$). However, a significant difference was seen between field-collected *PWW* rafts (0 of 40 low hatch) and rafts from *PWW* females isolated in vials (7 of 42 low hatch, $P < 0.05$). Thus, the low hatch exhibited by some rafts may have been introduced by handling procedures and not related to naturally occurring sterility in the *BrW* population or by matings with *PWW(I)* males.

4. The sterility of the *PWW(I)* males was verified each week by crossing subsamples with *PWC-79* females in small laboratory cages. An additional test was done using *BrW* females in a Quonset hut cage containing 2 chickens as a blood-meal source and a pond for oviposition (Terwedow et al. 1977). The egg rafts from these matings were evaluated as above for fertility. Hatch rates ranged from 0 to 9%, and embryonation rates from 0 to 27%. Thus, in assessing rafts from field-collected or Quonset hut females, those rafts with less than 15% hatch and 30% embryonation were attributed to matings with the *PWW(I)* males. Similar sterility ranges for comparable radiation doses were reported by Ainsley et al. (1980).

5. The mating competitiveness of the *PWW(I)* males against *BrW* males for *BrW*

females was estimated by releasing 1,000 adults of each genotype into a Quonset hut cage. Seven of 16 rafts exhibited low hatch and were attributed to matings with *PWW(I)* males. Based on this 7 to 9 ratio, the competitiveness of the *PWW(I)* males was 0.77. Since this value did not differ from 1.0 when tested by Chi-square ($P > 0.05$), the *PWW(I)* males were considered equally competitive. Similar competitiveness estimates were reported by Ainsley et al. (1980) using similar test conditions.

RESULTS

RELEASE AND RECAPTURE OF IRRADIATED MALES. From June 17 through August 28, 1980, a total of 72,747 *PWW(I)* males were irradiated and shipped to Bakersfield, of which 71,016 *PWW(I)* males (98%, mean = 5,910/wk) were released at Breckenridge. Of these, 25,501 were marked with fluorescent dust. The maximum weekly release was 12,949 *PWW(I)* males the week of July 19 (Fig. 2a).

A total of 162 (0.64%) dusted *PWW(I)* males were recaptured by all collection methods, 159 in Canyon B and 3 in Canyon C. The proportions of dusted *PWW(I)* males among all males collected in CO_2 -light traps did not differ significantly between Canyons B and C ($P > 0.05$, Table 1). None were recaptured in Canyon A.

The estimated daily survival rate for *PWW(I)* males recaptured in Canyon B was 82%. Assuming survivorship to be constant throughout, the number of *PWW(I)* males remaining in Canyon B was estimated daily and peaked at 10,255 during the week ending July 26 (Fig. 2a). The proportion of *PWW(I)* males among all males in Canyon B was estimated from the pooled recaptures of dusted *PWW(I)* males in light trap, shelter and swarm collections and never exceeded 0.241 (Fig. 2b).

PWW(I) males seemed to behave similarly to *BrW* males. The proportion of sterile males among all males in Canyon B was 0.082 in light trap collections, 0.060

in shelters and 0.068 in swarms. Assuming that mating in *Cx. tarsalis* is associated with swarming, the present results indicated that the *PWW(I)* males intermingled with the *BrW* population and may have been at the proper place at the proper time to compete for females from the target population.

INDUCTION OF STERILITY. A total of 2,422 egg rafts was oviposited by unmarked *BrW* females collected by light traps in Canyons A, B and C after release of the *PWW(I)* males. Of these egg rafts, 224 (9%) exhibited characteristic low hatch and may have come from matings with *PWW(I)* males. Unexpectedly, the proportion of low-hatch rafts among all rafts did not differ significantly among the 3 Canyons (Table 1). In Canyon B,

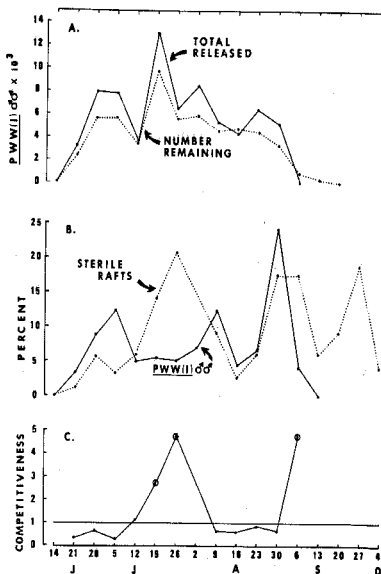


Fig. 2. Weekly changes in (A) total *PWW(I)* males released per week and number remaining in Canyon B on the last day of each week; (B) proportion of *PWW(I)* males among all males and proportion of sterile egg rafts among all rafts; and (C) calculated mating competitiveness (circled points were significantly greater than 1.0, $P < 0.05$).

the proportion of low hatch rafts after *PWW(I)* male releases began (10.0%) was significantly greater than the 2.9% pre-release sterility ($P < 0.05$). Similar results were noted during the pilot release at Breckenridge (Asman et al. 1980).

Temporally, increases in the proportion of sterile rafts generally paralleled increases in the number of *PWW(I)* males (Figures 2a and 2b). The final increase in the proportion of sterile rafts in late September was attributed to the relatively high proportion of older non-diapausing females persisting in CO_2 -light trap collections (Nelson 1964).

COMPETITIVENESS OF *PWW(I)* MALES. Estimates of the mating competitiveness of the *PWW(I)* males were calculated weekly for Canyon B and ranged from a low of 0.085 the week of June 21 to a high of 4.82 the weeks of July 25 and September 5 (Fig. 2c). The lower competitiveness estimates occurred early during the releases and were attributed, in part, to the survival of *BrW* females inseminated prior to the release of *PWW(I)* males.

Overall, competitiveness was 1.1, not significantly different from 1.0 ($P > 0.05$). In addition, 12 (18%) of 68 recaptured *BrW* females, that were marked and released as virgins during mark-release studies, oviposited low hatch rafts. The present results suggested that the *PWW(I)* males successfully competed against the *BrW* males for marked *BrW* females in nature.

DYNAMICS OF THE TARGET POPULATION. Despite the introduction of 71,016 competitive *PWW(I)* males, the temporal relative abundance pattern of the target population in Canyon B was similar to those in adjacent Canyons A and C (Figure 3). In fact, light trap abundance levels in Canyon B were higher than Canyons A and C throughout the study. The slight bimodality in the Canyon B curve was attributed to normal vernal and autumnal increases in abundance (Fine et al. 1979) and not necessarily to the release of *PWW(I)* males.

A total of 12,303 *BrW* females and 9,655 *BrW* males were collected as pupae

Table 1. *Culex tarsalis* collections and sterile raft occurrence in each canyon during sterile male release and mark-release-recapture studies at Breckenridge, Kern County, 1980.¹

	Canyon A	Canyon B	Canyon C
Light trap collections			
Sterile male release period, June 18–Sept 13			
Marked <i>PWW(I)</i> ♂♂	0	31	3
Unmarked ♂♂	109	1,101	129
Marked <i>PWW(I)</i> ♂♂/total ♂♂	0.000	0.027	0.023
Proportion <i>PWW(I)</i> ♂♂ ²	0.000	0.082	0.068
Unmarked <i>BrW</i> ♀♀	4,701	19,156	6,133
5 mark-release-recapture periods, May 30–Sept 28			
Marked <i>BrW</i> ♂♂	0	30	0
Unmarked ♂♂	80	691	83
Marked <i>BrW</i> ♂♂/total ♂♂	0.000	0.042	0.000
Marked <i>BrW</i> ♀♀	46	540	150
Unmarked ♀♀	5,087	17,496	6,896
Marked <i>BrW</i> ♀♀/total ♀♀	0.009	0.030	0.021
Sterility			
Egg rafts examined	505	1,309	608
Low hatch rafts	42	131	51
Proportion <i>PWW(I)</i> ♂ matings ³	0.054	0.071	0.055

¹—Genotype designations are defined in the methods section.

²—(3x marked *PWW(I)* ♂♂)/total ♂♂.

³—Low hatch rafts/total −0.029 background sterility.

at Breckenridge, allowed to emerge in the laboratory, dusted and then released. Recaptures from Canyon B totaled 595 (4.8%) females and 171 (1.8%) males. Although light traps caught more total females than males (18,036 females to 721 males) shelter and swarm collections included more males than females (2,427 males to 1,544 females and 1,341 males to 25 females, respectively). The proportions of marked/total were comparable for all 3 methods (females:males in light traps, 0.030:0.042; shelters, 0.035:0.052; and swarms, 0.042:0.012). These data indicated that the released *BrW* adults intermingled well with the Canyon B population. Marked *BrW* males were not recaptured in either Canyons A or C (Table 1). Significantly higher pro-

portions of marked females were recaptured in Canyon C than in Canyon A ($P < 0.001$, Table 1) which was attributed to the topography of the study site; i.e., Canyon A was farther from Canyon B and separated by higher ridges than was Canyon C (Figure 1). Emigrants from Canyon B were presumed responsible for most of the rafts with low hatch from females collected in Canyons A and C.

The proportion of dusted *BrW* males recaptured was significantly greater than that of dusted *PWW(I)* males ($P < 0.001$), due to increased sampling effort during mark-release-recapture experiments. However, the 2 strains exhibited similar dispersion patterns among the 3 canyons (Table 1). The estimated loss rate for *PWW(I)* males (18%) was comparable to

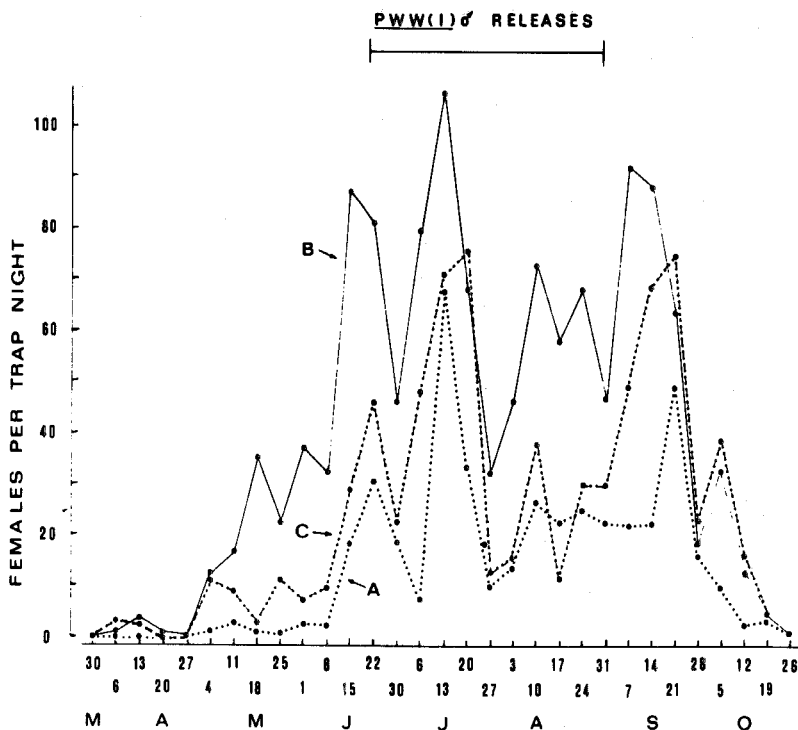


Fig. 3. Weekly changes in female relative abundance (females per CO_2 -light trap night) in Canyons A, B and C at Breckenridge, 1980.

the loss rate of *BrW* males during July and August (29% and 19%, respectively).

Absolute female population size in Canyon B increased during July and August after the initiation of *PWW(I)* male releases (Table 2). The estimated weekly female additions in Canyon B (7 times the mean additions per day in each mark-release experiment, Table 2) exceeded the total weekly releases of *PWW(I)* males throughout the summer (Figure 2a). Similar trends presumably occurred throughout the study for the *BrW* male population, although this could not be determined quantitatively due to the inadequate sampling of males. Also, additions to Canyon B included some immigrant females which had mated in Canyons A or C, away from concentrations of *PWW(I)* males.

DISCUSSION

The rationale of utilizing a natural population as a source of males for radiosterilization and release into a smaller target population was sound and circumvented the poor mating competitiveness of laboratory-adapted strains reported by Asman et al. (1979) and Milby et al. (1980). The *PWW(I)* males dispersed from the release site, intermingled with the *BrW* population and competed well against the *BrW* males for *BrW* females under both Quonset hut and field conditions. The significant increase in the

incidence of low hatch rafts after *PWW(I)* male release indicated that sterility was induced in the Canyon B population. Similar trends were observed during the pilot study (Asman et al. 1980).

The present study did not achieve population suppression. Sufficiently large concentrations of *PWW* immatures were difficult to locate consistently despite repeated and extensive searches of the Poso West area and too few *PWW(I)* males were released throughout the study. In fact, the estimated 69,630 *BrW* females added to the population by emergence or immigration during the August 14–24 mark-release experiment was comparable to the 71,016 *PWW(I)* males released during the entire experiment. In addition, the first *PWW(I)* males were released after the *BrW* population had completed the vernal increase and a relatively large population was established. Our inability to consistently procure sufficient numbers of *PWW(I)* males for release from Poso West (a well-studied, large population) suggested the use of field-collected material would not be practical for future studies, even though it circumvented reduced mating competitiveness and precluded the need for mass production.

A new colony of *Cx. tarsalis* from Breckenridge was established in large cages (70 × 70 × 70 cm) during autumn, 1980. This colony will be expanded during winter and large numbers of irradiated males from this source will be released at

Table 2. *Culex tarsalis* loss and addition rates and population size estimates during mark-release-recapture studies at Canyon B, Breckenridge, Kern County, 1980.

Estimated parameters	Mark-release-recapture periods				
	May 30– June 9	June 20– July 1	July 17– July 27	Aug 14– Aug 24	Sept 15– Sept 28
Female daily loss rate	0.26	0.25 ¹	0.37	0.13	0.24
Male daily loss rate	N.E. ²	N.E.	0.29	0.19	N.E.
Mean female population size	3,936	15,990	18,402	49,122	8,182
Standard deviation	899	2,209	1,323	7,015	739
Mean females added daily	1,157	2,149	1,896	6,963	1,470
Female addition rate	0.29	0.13	0.10	0.14	0.18

¹—Non-significant regression estimate ($P > 0.05$).

²—N.E. = not estimable.

Breckenridge beginning early April 1981. Sterile males should exceed the abundance of *BrW* males in early spring, thus facilitating a suppression of the vernal rise of the *BrW* population. If the majority of the first generation females mate with sterile males, the midsummer population may be substantially reduced based on the predictive calculations of Moon (1976). Hopefully, the utilization of recently colonized males (<10 generations in the laboratory) will minimize the loss of mating competitiveness observed in previous releases employing laboratory-adapted strains.

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