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EFFECTS OF TRAP DESIGN AND CO_2 PRESENTATION ON THE MEASUREMENT OF ADULT MOSQUITO ABUNDANCE USING CENTERS FOR DISEASE CONTROL–STYLE MINIATURE LIGHT TRAPS

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ABSTRACT. Centers for Disease Control miniature light traps augmented with CO_2 provide an effective method of monitoring *Culex* abundance and may provide a useful supplement to New Jersey light traps used by the California Mosquito Surveillance Program. To assist in standardizing sampling protocols, the present research compared the catch of adult mosquitoes collected using 4 trap designs and 3 CO_2 presentation methods. When augmented with dry ice, the Arbovirus Field Station (AFS) trap (consisting of a 3-in. fan mounted into a white polyvinyl chloride pipe and operated without a light source or rain shield) collected as many or more *Culex* females than similar traps purchased from John W. Hock and American Biophysics, or a trap with a 4.25-in. 2-bladed fan constructed by the Orange County Vector Control District (similar to the Encephalitis Virus Surveillance model distributed by Bioquip). Few blooded or gravid females and males were collected, indicating that CO_2 released from the dry ice and not light probably was the primary attractant. Catch of *Culex tarsalis* females in traps baited with CO_2 released at 0.5–1.5 liters/min from gas cylinders was significantly greater than in traps baited with dry ice, even though the CO_2 release rates from the dry ice at dusk probably were comparable to that released from the cylinders and averaged 0.4–0.5 liters/min for the night. Traps baited with 0.5 liters/min of CO_2 gas released in 15 3- or 2-sec bursts per hour collected the fewest mosquitoes. In all experiments, trap location effects were significant and accounted for as much variability in catch size as trap design or CO_2 presentation. Sampling efficiency of all trap designs or CO_2 presentations were consistent over time, space, and different levels of mosquito abundance.

KEY WORDS Adult mosquito sampling, CO_2 attractant, *Culex*, California

INTRODUCTION

Vector abundance measurements comprise an important component of mosquito surveillance and control programs. Recent research (Reisen et al. 1999) confirmed previous studies (Milby and Reeves 1989) that throughout the major biomes of California, Centers for Disease Control (CDC)-(Sudia and Chamberlain 1962) or Encephalitis Virus Surveillance (EVS)-style traps (Pfuntner 1979, Rohe and Fall 1979) baited with dry ice (hereafter, CO_2 traps) catch significantly more female *Culex tarsalis* Coquillett than do New Jersey (NJ) light traps or gravid female traps baited with a bulrush infusion. Therefore, a systematic CO_2 trapping program may provide a useful supplement to the existing California Mosquito Surveillance Program that relies solely on NJ light traps.

Mosquito control agencies in California employ a variety of commercially produced and locally constructed CO_2 traps, operate traps with or without light, and present the CO_2 bait in a variety of containers, including metal or plastic 1-gal (3.8-liter) paint buckets with four 0.25-in. (0.6-cm) holes drilled into the sides or bottom and Styrofoam shipping containers. Before a statewide CO_2 trapping program can be initiated, we believed that it was necessary to measure the impact of different CO_2 trap designs and methods of CO_2 presentation on mosquito abundance estimates.

Basically, 2 types of CO_2 traps were used: the CDC-style trap with a 3-in. (7.62-cm) 4-bladed fan and a small lamp positioned under a 1-ft (30.5-cm)-diameter rain shield (Sudia and Chamberlain 1962), and the EVS-style trap with a 4.5-in. (11.4-cm) 2-bladed fan and a small light positioned under a 5-in. (12.7-cm) lid containing the batteries and electrical circuits (Pfuntner 1979, Rohe and Fall 1979). Both traps come with a variety of features, including photocells to turn the trap on and off, damper lids to prevent the loss of catch if the fan fails or the trap is turned off, air deflectors to prevent the desiccation of the mosquitoes, and rechargeable gel-cell or disposable D-cell power sources. Regardless of trap design, augmentation with CO_2 subliming from dry ice markedly improves catch size (Newhouse et al. 1966). Although *Cx. tarsalis* feeds primarily on birds (Reisen and Reeves 1990), the number of females collected in traps increased as a function of the amount of CO_2 released, from 0.025 (simulating a chicken) to 2.5 (a young cow) liters/min (Reeves 1953). The sublimation rate of dry ice changes markedly as a function of dry ice type (pellet or solid block), quantity, and ambient temperature. At 27°C, for example, the sublimation rate from 1 kg of dry ice decreased from 1.0 to 0.4 liters/min over a simulated 12-h trapping period (Pfuntner et al. 1988).

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The present study compared the ability of 4 trap designs and 3 CO_2 presentation methods to collect *Culex* mosquitoes. The engineering features of the 4 trap designs including air discharge and power requirements were described elsewhere (Cummings and Meyer 1999). Specific hypotheses tested included: trap design alters catch size more than time and space effects, a constant release rate of CO_2 from bottled gas catches more mosquitoes than a changing CO_2 release rate from dry ice, and pulsing CO_2 to simulate emanations from a respiring host collects more host-seeking females than a constant release rate.

MATERIALS AND METHODS

Our hypotheses were tested during 3 experiments that were conducted during the summer of 1998.

Experiment 1 was done between June 30 and July 23 at the Tracy Ranch in Kern County (35°25′N, 119°24′W) along a corridor of riparian vegetation (willows [*Salix*], gum [*Eucalyptus*], spear scale [*Atriplex*]) at Jerry Slough that transected a monoculture of irrigated cotton. Experiment 1 compared the catch of host-seeking *Cx. tarsalis* females in 4 types of traps operated according to manufacturer instructions.

The Arbovirus Field Station (AFS) trap was a CDC-style trap constructed by AFS personnel and consisted of a white 3.5-in.-diameter polyvinyl chloride (PVC) pipe containing a 3-in.-diameter 4-bladed fan attached to a Mabuchi® motor (purchased from John W. Hock Company, Gainesville, FL) that was powered by a rechargeable 6-V 8-ampere-hour Eagle-Picher® battery. This trap did not contain a light source or a rain shield.

The Orange County Vector Control District (OCVCD) trap was modified from the previous EVS trap design (Pfuntner 1979), constructed by OCVCD personnel, and consisted of a 4.25-in. 2-bladed fan set into a black acrylonitrile–butadiene–styrene cylinder. A small light was positioned under the 5-in.-diameter lid that contained the electrical circuitry and 3 1.5-V D-cell batteries.

The CDC traps manufactured by American Biophysics (ABC trap kit, American Biophysics Corp., East Greenwich, RI) and Hock (Model 1012 miniature light trap, John W. Hock Company) companies contained a 3-in.-diameter, 4-bladed fan inserted into a PVC or plexiglass cylinder and were covered with a rain shield. Traps were operated with the small 6-V lamp on. The American Biophysics trap had a blue and white thermos to hold the dry ice that was inserted into the rain shield; a small tube released the CO_2 gas at 0.5 liters/min under the rain shield above the fan. Both models had a photocell that turned the trap motors and lamps on at sunset.

Traps were suspended 1.5-m high from similar fence posts, operated from afternoon (1500–1600 h) until morning (0600–0800 h Pacific Standard Time), and baited with a 3-lb (1.5-kg) block of dry ice. Dry ice was placed in the American Biophysics thermos, a 1-gal plastic paint can for the OCVCD trap, and in Styrofoam holders (petri dish mailers from Polyfoam Packers, Wheeling, IL) for the Hock and AFS traps. As specified in the instructions, the OCVCD trap was suspended under the paint can, whereas the AFS and Hock traps were positioned adjacent to the Styrofoam holder. The 4 traps in experiment 1 were replicated along 2 parallel transects on the east and west sides of Jerry Slough (8 traps per night), operated 2 times per week for 4 wk, and rotated through the 4 trap positions twice (8 nights).

Experiment 2 was done from June 23 through September 9 at the San Joaquin Marsh in Orange County (33°45′N, 117°52′W) along access roads through dense stands of cattails (*Typha*) and riparian vegetation dominated by willows. Experiment 2 compared the same 4 types of traps as experiment 1, except that they each were baited with 3 lb of pelletized dry ice in 1-gal paint cans (except for the Biophysics trap where the ice was placed in the thermos). The 4 trap types were replicated along 2 transects (8 traps per night), operated on 2 consecutive nights per week (12 wk), and rotated through the 4 trap positions 6 times. During each week, traps were operated the night before and during Scourge® (AgrEvo, Montvale, NJ) ground ultra-low-volume applications (resmethrin at 8 fluid oz/acre) that commenced 0.5 h after sunset.

Experiment 3 was conducted from August 31 through October 7 at the Kern River in Kern County (35°14′N, 119°14′W) along brush (spear scale, nettles [*Urtica*]) and riparian (cottonwood [*Populus*], willows) vegetation surrounding an old field dominated by salt grass (*Dystilichilis*). Experiment 3 compared the effects of 3 CO_2 presentations on mosquito catch in AFS traps. Dry ice (1.5-kg blocks) was placed in Styrofoam holders and in 1-gal plastic buckets with 4 0.25-in. holes drilled 3 in. from the bucket lid, which was made from a PVC pipe test cap. Holders were weighed before and after deployment to estimate the quantity of CO_2 gas released. The CO_2 gas was dispensed from tanks equipped with regulators and flow meters (Gilmont Instruments, Inc., Barrington IL) set to release constant volumes of 0.5, 1.0, and 1.5 liters/min through a diffuser made from a perforated plastic petri dish capping a 6-cm-diameter plastic funnel. To simulate respiration, 0.5 liters/min of CO_2 gas was pulsed 15 times per minute using either 2:2 or 3:1 sec on : off cycles. The entire pulsing unit was purchased from American Biophysics Corp. and consisted of a preset regulator, a CO_2 nozzle with a fixed aperture to release 0.5 liters/min, and a solenoid pulsing system (CONTROL1). All CO_2 sources were suspended (dry ice holders) or released (diffuser) adjacent (ca. 10-cm distance) to the entrance of an AFS trap.

In all experiments, mosquitoes were returned to the laboratory and enumerated by species, sex, and female abdominal condition (empty, bloodfed, or gravid). Counts were transformed by $\ln(y + 1)$ and tested by multiway analysis of variance (ANOVA) using NCSS 2000 software (Hintze 1998). Nonsignificant interaction effects were pooled with the error term. Means within main effects were compared by posteriori least significant range tests ($\alpha = 0.05$). Means presented in the text or figures were back-transformed geometric means.

RESULTS

Experiment 1

A total of 3,056 female mosquitoes was collected during 64 trap nights at the Tracy Ranch, of which 95% were *Cx. tarsalis.* Of these, 97% were empty and presumed to be host-seeking. Only 18 males were collected, 13 of which were collected in the Hock trap. The AFS trap collected significantly ($P < 0.01$) more *Cx. tarsalis* females than the 3 other traps (Fig. 1), and this relationship remained consistent over both time and space, because the 1st-order interaction terms were not significant ($P > 0.05$). Catch decreased significantly ($P < 0.01$) over the 4 wk study, and traps operated along transect 1 collected significantly ($P < 0.01$) more mosquitoes than transect 2 (Fig. 1). Trap type accounted for 9% of the total variance in the ANOVA, compared to 56% for weeks and 13% for transects (Fig. 1).

Experiment 2

A total of 37,141 females was collected in the San Joaquin Marsh in Orange County during 188 trap nights, of which 40 and 58% were *Cx. tarsalis* and *Culex erythrothorax* Dyar, respectively. Again, most females were empty (>99%) and presumed to be host-seeking. A total of 408 male *Cx. tarsalis* were collected: 99 by the Hock trap, 149 by the Biophysics trap, 104 by the OCVCD trap, and 56 by the AFS trap operated without light. Consistent with the results of experiment 1, the AFS trap collected significantly ($P < 0.001$) more *Cx. tarsalis* females than the remaining traps (Fig. 2). Trap performance remained consistent over time (wks), space (trap sites), and abundance (prespray vs. spray nights), because all 1st-order interaction effects in the multiway ANOVA were not significant ($P > 0.05$). Catch size decreased significantly ($P < 0.001$) over time (wks, Fig. 2) and varied significantly ($P = 0.004$) among trap sites (range, 27.6–53.2 females/trap night). The application of resmethrin significantly ($P < 0.001$) reduced host-seeking abundance from a mean of 78.8 females per trap night prespray to 21.0 females per trap night on the night of spray (73% reduction). Despite 3 subsequent resmethrin applications each week, abundance recovered quickly and usually

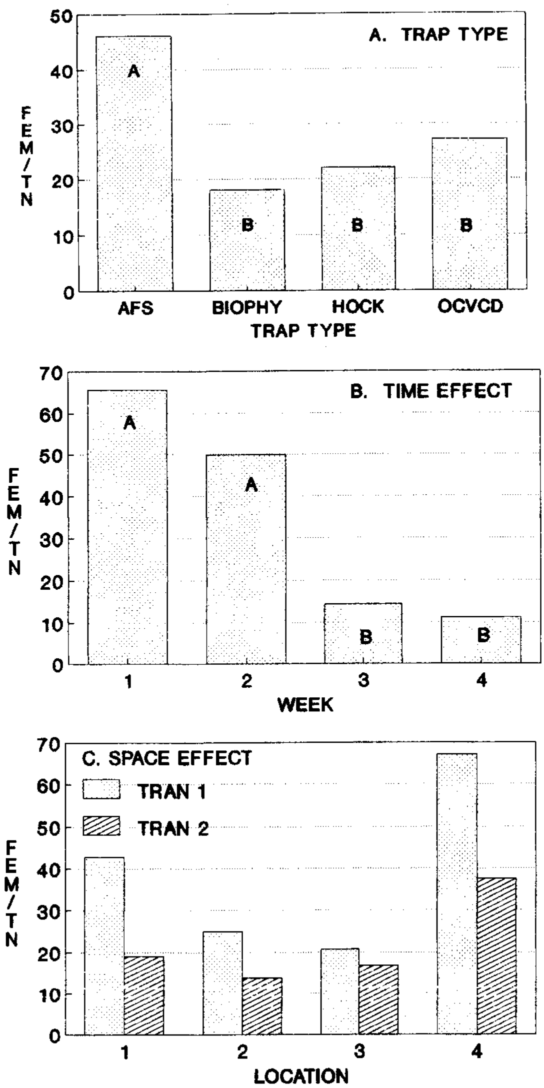

Fig. 1. Effects of (A) trap design (AFS, Arbovirus Field Station; BIOPHY, American Biophysics; HOCK, Hock miniature; OCVCD, Encephalitis Virus Surveillance trap), (B) time (wks), and (C) space (trap location and transects) on the catch of *Culex tarsalis* females at Tracy Ranch, Kern County, CA, 1998. Bars within panels with the same letter were not significantly different when tested by a least significant range test ($P > 0.05$).

reached prespray levels by the following week (Fig. 2). Overall, time (wks) accounted for 39% of the variability in catch size in the ANOVA, followed by spray (27%), trap type (11%), and trap sites (3%).

Trends for *Cx. erythrothorax* differed somewhat from *Cx. tarsalis.* The Hock and AFS traps collected the most females, followed by the OCVCD and Biophysics traps (Fig. 2). Again, this pattern was consistent over time, space, and abundance, because the 1st-order interaction effects in the AN-

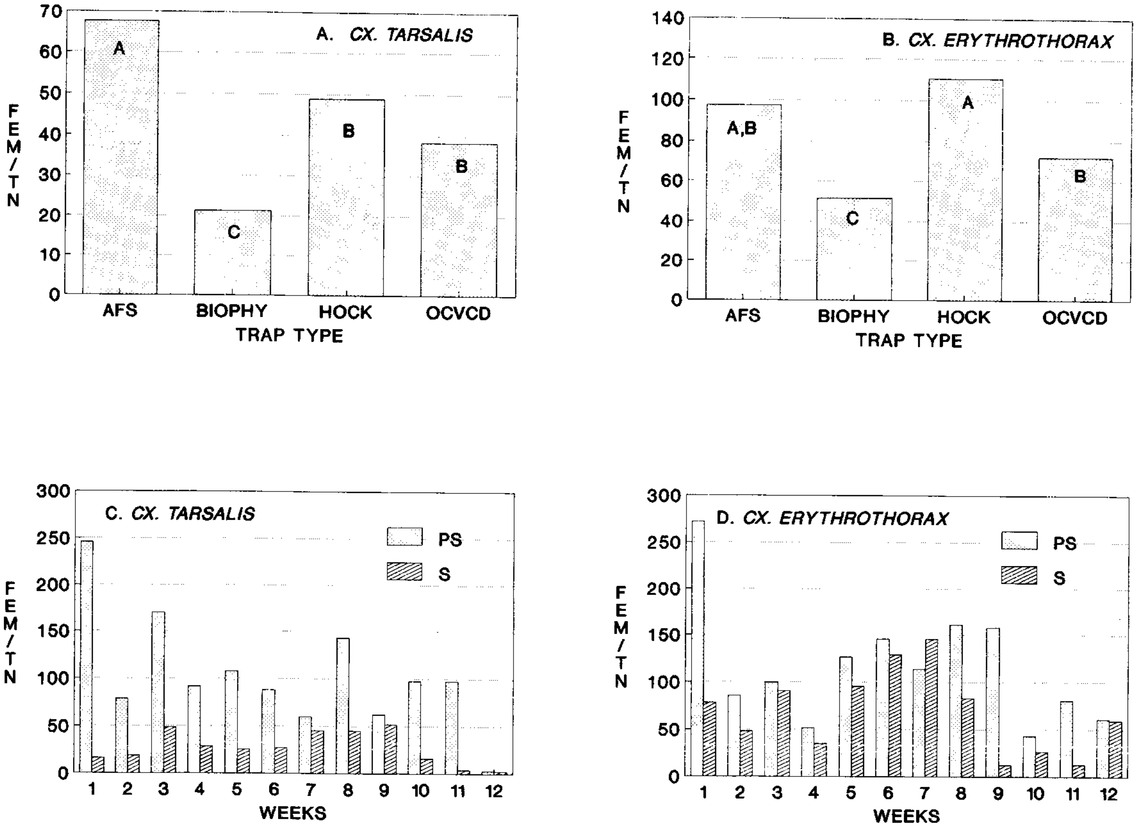

Fig. 2. Effects of trap type (see Fig. 1), time (wks), and resmethrin application (PS, prespray; S, spray night) on the catch of *Culex tarsalis* and *Culex erythrothorax* at San Joaquin Marsh, Orange County, CA, 1998.

OVA were not significant ($P > 0.05$). Catch decreased significantly ($P < 0.001$) over time (wks, Fig. 2) and varied significantly ($P < 0.001$) among trap sites (range, 47.6–127.0 females/trap night). Applications of resmethrin significantly ($P < 0.001$) reduced host-seeking abundance from a mean of 102.8 females per trap night prespray to 61.6 females per trap night during the night of spray (40% reduction). Catch recovered quickly and usually reached prespray abundance by the following week (Fig. 2). Spray effectiveness varied significantly over time (Fig. 2) as indicated by the week by spray interaction term in the ANOVA ($P = 0.003$). Overall, time (wks) accounted for 31% of the variability in catch size in the ANOVA, followed by trap site (11%), trap type (9%), and spray (7%).

Experiment 3

A total of 18,279 female mosquitoes was collected during 71 trap nights at the Kern River, of which 95% were *Cx. tarsalis.* Of these, 99% were empty and presumably host-seeking. No males were collected. Within the range of CO_2 concentrations used during the present study, devices that delivered the highest CO_2 release rates collected the most *Cx. tarsalis* (Fig. 3). Constant CO_2 gas released from cylinders collected more females than dry ice presented in either type of holder, and all of these methods collected more females than the pulsing unit equipped with a 0.5-liter/min release aperture. Even when comparison was restricted to the constant release rate of 0.5 liters/min, the pulsing unit collected significantly fewer females ($P < 0.01$). The white Styrofoam holder retained dry ice better than the gray-colored bucket with a black lid, and therefore released on average less CO_2 (ca. 350 liters/16 h or 0.4 liters/min vs. 450 liters/16 h or 0.5 liters/min, respectively). However, when traps baited with these holders were compared statistically, no difference was found in the mean numbers of females collected ($P = 0.61$). Also, no significant difference ($P = 0.36$) was found in catch among traps baited with constant release rates of CO_2 gas ranging from 0.5 to 1.5 liters/min (Fig. 3). Catch at traps baited with CO_2 gas released at 0.5 liters/min collected as many or more *Cx. tarsalis* females than traps baited with dry ice in either holder, even though the release rate from these holders probably equaled or exceeded the gas release rate at dusk. Both time ($P < 0.001$) and space

(P = 0.05) effects were significant in the ANOVA (Fig. 3). However, because of the wide range in catch size (11–263 females/trap night) due mostly to the low catch by the pulsing unit, CO_2 presentation accounted for 47% of the total variance in the ANOVA, followed by time (26%) and space (4%).

DISCUSSION

Trap design and CO_2 presentation significantly affected the measurement of mosquito abundance in all 3 experiments. The AFS trap operated without a light source collected more or as many *Culex* females as the 3 other traps, indicating that CO_2 was the principal attractant. Few males were collected during experiments 1 and 2, and, therefore, a light source would seem unimportant or even deleterious, considering the increased drain on the power source (Cummings and Meyer 1999). These results agreed with previous comparisons (Reisen et al. 1983). Deleting the light source also facilitated processing by eliminating unwanted phototactic insects such as chironomids. Most likely, the AFS trap collected more mosquitoes because it was operated without a rain shield (Hock and Biophysics traps) or power unit (OCVCD trap) over the trap entrance, thereby increasing trap access to host-seeking mosquitoes. Fan speed and trap discharge were actually less than the Hock trap and comparable to the OCVCD trap (Cummings and Meyer 1999). Poor performance by the Biophysics trap was attributed to the sealed dry ice unit that discharged the sublimed CO_2 gas at the rate of 0.5 liters/min through a tube that opened directly above the trap entrance and to the constrictive metal screen placed over the trap entrance.

Traps baited with CO_2 gas released at a constant rate from cylinders collected significantly more *Cx. tarsalis* females than traps baited with dry ice, even though the estimated CO_2 gas release rate from the dry ice holders at dusk (probably $>$0.8 liters/min) was comparable to or greater than the 0.5–1.5 liters/min released from the cylinders. *Culex tarsalis* typically commences host-seeking at sunset and terminates activity before midnight (Reisen et al. 1997). Therefore, unless this behavioral pattern was modified at the Kern River during 1998 and host-seeking continued into the morning hours after most of the dry ice had dissipated, it was difficult to interpret why traps baited with dry ice caught fewer mosquitoes than traps baited with the gas from cylinders.

Pulsing CO_2 gas released at 0.5 liters/min during 15 4-sec cycles of 2 sec on : 2 sec off or 3 sec on : 1 sec off was not a suitable attractant for host-seeking *Cx. tarsalis* females when compared with either dry ice or gas from cylinders released at similar rates. Although the aperture released CO_2 gas at the rate of 0.5 liters/min, the actual volume of gas released over time was reduced by 50% for the 2:2 cycle

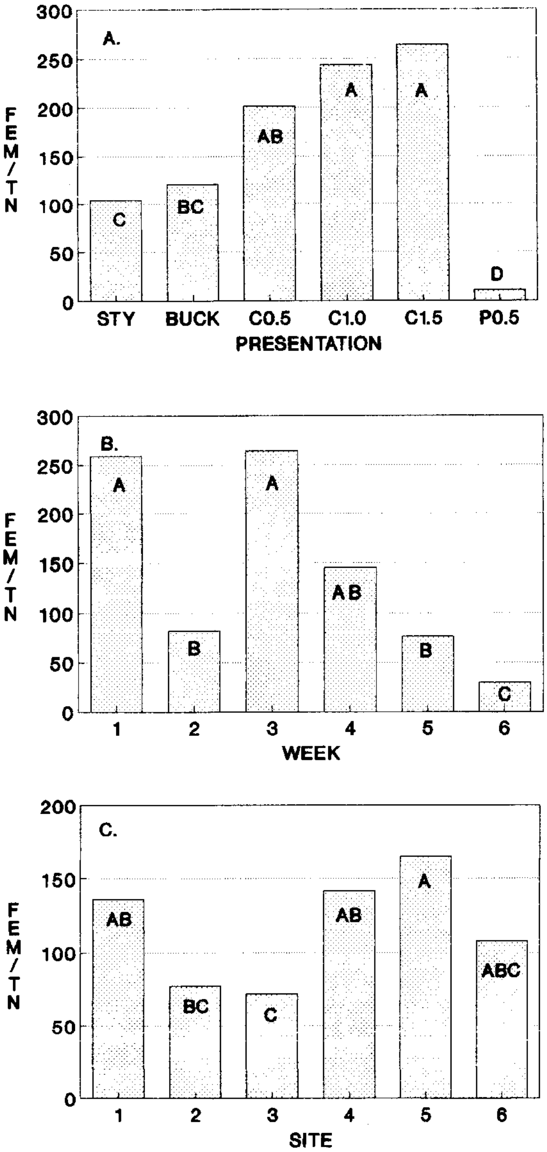

Fig. 3. Effects of (A) CO_2 gas presentation (dry ice in Styrofoam [STY] or bucket [BUCK]; bottled gas released at a constant rate [C] of 0.5, 1.0, or 1.5 liters/min or pulsed [P] at 0.5 liters/min), (B) time (wks), and (C) space (trap site) on the catch of *Culex tarsalis* females in Arbovirus Field Station traps at the Kern River, Kern County, CA, 1998.

and by 25% for 3:1 cycle. Pulsing the CO_2 gas theoretically precluded summation effects on females exposed to a constant stream of CO_2 gas. However, variability of terrain features and wind movement most likely created irregularities in the flow of CO_2 gas, even when released from a cylinder at a constant rate.

In all 3 experiments, trap site effects in the ANOVAs were significant and accounted for greater or comparable variability as did trap design or CO_2 presentation method effects (excluding the pulsing unit). Site effects were least in San Joaquin Marsh and greatest at Tracy Ranch, which probably reflected the relative degree of habitat heterogeneity. At San Joaquin Marsh, traps were deployed along roads that formed transects through relatively homogenous habitat. In contrast, Jerry Slough meandered through a large cotton ranch that provided a heterogeneous ecotonal interface. Further research is needed in California to extend our understanding of mosquito flight patterns in relation to landscape features (Bidlingmayer and Hem 1981).

The current evaluation of trap design coupled with the recent engineering analysis (Cummings and Meyer 1999) indicated that a relatively simple trap design collected as many or more mosquitoes than more complicated designs. Dry ice was less effective than bottled gas as an attractant for *Cx. tarsalis* females; however, cost, safety during transport, and portability favor use of dry ice in routine monitoring programs. Decisions on trap placement were shown to be an important consideration in developing a suitable sampling program for mosquito surveillance. On-going research is attempting to quantify the ecological characteristics of productive trap locations to reduce microhabitat effects in mosquito sampling.

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REFERENCES CITED

Bidlingmayer WL, Hem DG. 1981. Mosquito flight paths in relation to the environment. Effect of the forest edge upon trap catches in the field. *Mosq News* 41:55–59.

Cummings RF, Meyer RP. 1999. Comparison of the physical parameters of 4 types of modified CDC-style traps in reference to their mosquito collecting efficiency. *Proc Mosq Vector Control Assoc Calif* 67: (in press).

Hintze JL. 1998. *NCSS Statistical Software (computer program). 2000* Kaysville, UT: NCSS.

Milby MM, Reeves WC. 1989. Comparison of New Jersey light traps and CO_2-baited traps in urban and rural areas. *Proc Calif Mosq Vector Control Assoc* 57:73–79.

Newhouse VF, Chamberlain RW, Johnston JG Jr, Sudia WD. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. *Mosq News* 26:30–35.

Pfuntner AR. 1979. A modified CO_2-baited miniature surveillance trap. *Bull Soc Vector Ecol* 4:31–35.

Pfuntner AR, Reisen WK, Dhillon MS. 1988. Vertical distribution and response of *Culex* mosquitoes to differing concentrations of carbon dioxide. *Proc Calif Mosq Vector Control Assoc* 56:69–74.

Reeves WC. 1953. Quantitative field studies on the carbon dioxide chemotropism of mosquitoes. *Am J Trop Med Hyg* 2:325–331.

Reisen WK, Reeves WC. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. In: Reeves WC, ed. *Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987* Sacramento, CA: California Mosquito and Vector Control Association. p 254–329.

Reisen WK, Lothrop HD, Meyer RP. 1997. Time of host-seeking by *Culex tarsalis* (Diptera: Culicidae) in California. *J Med Entomol* 34:430–437.

Reisen WK, Milby MM, Reeves WC, Meyer RC, Bock ME. 1983. Population ecology of *Culex tarsalis* (Diptera:Culicidae) in a foothill environment of Kern County, California: temporal changes in female relative abundance, reproductive status, and survivorship. *Ann Entomol Soc Am* 76:800–808.

Reisen WK, Boyce K, Cummings RF, Delgado O, Gutierrez A, Meyer RP, Scott TW. 1999. Comparative effectiveness of three adult mosquito sampling methods in habitats representative of four different biomes of California. *J Am Mosq Control Assoc* 15:24–31.

Rohe DL, Fall RP. 1979. A miniature battery powered CO_2 baited trap for mosquito borne encephalitis surveillance. *Bull Soc Vector Ecol* 4:24–27.

Sudia WD, Chamberlain RW. 1962. Battery-operated light trap, an improved model. *Mosq News* 22:126–129.