MOSQUITO MANAGEMENT ON NATIONAL WILDLIFE REFUGES ECO\$YSTEM EFFECTS STUDY

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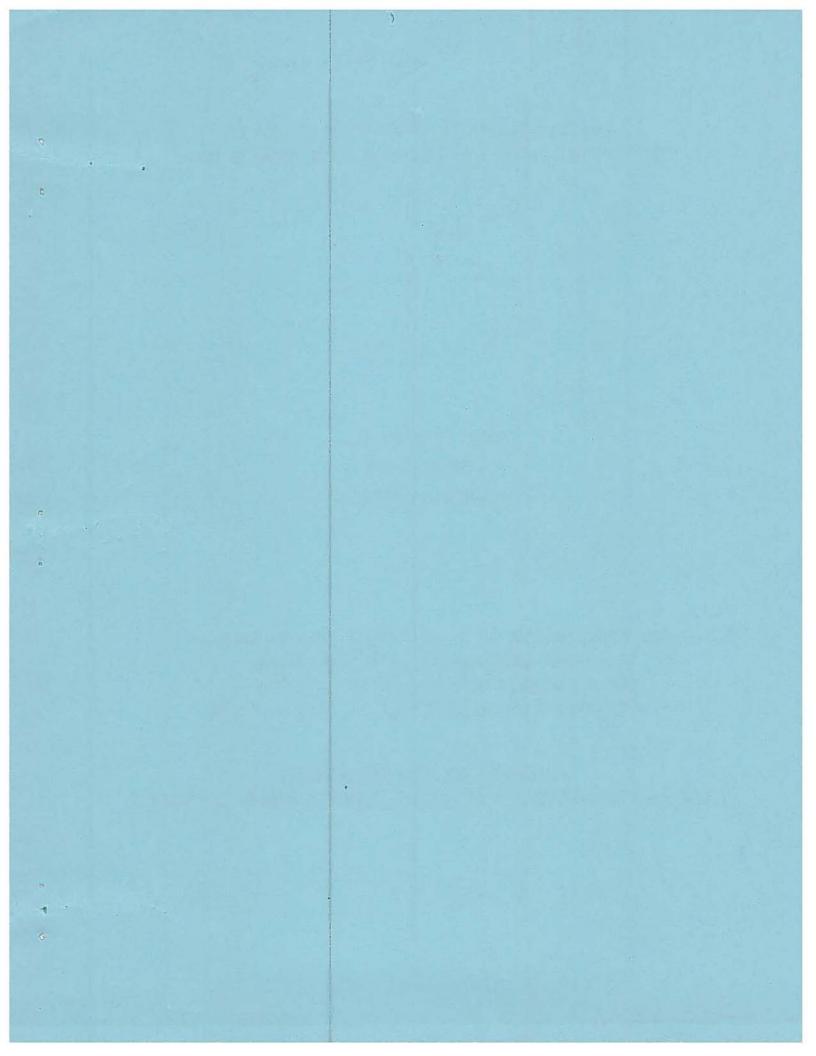
Phase II, Part 1 - Effects of Ultra Low Volume Applications of Pyrethrin, Malathion and Permethrin on Macro-Invertebrates in the Sacramento National Wildlife Refuge, California

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Mosquito Management on National Wildlife Refuges Ecosystem Effects Study: Phase II - California. Effects of ultra low volume applications of pyrethrin, malathion and permethrin on macro-invertebrates in the Sacramento National Wildlife Refuge

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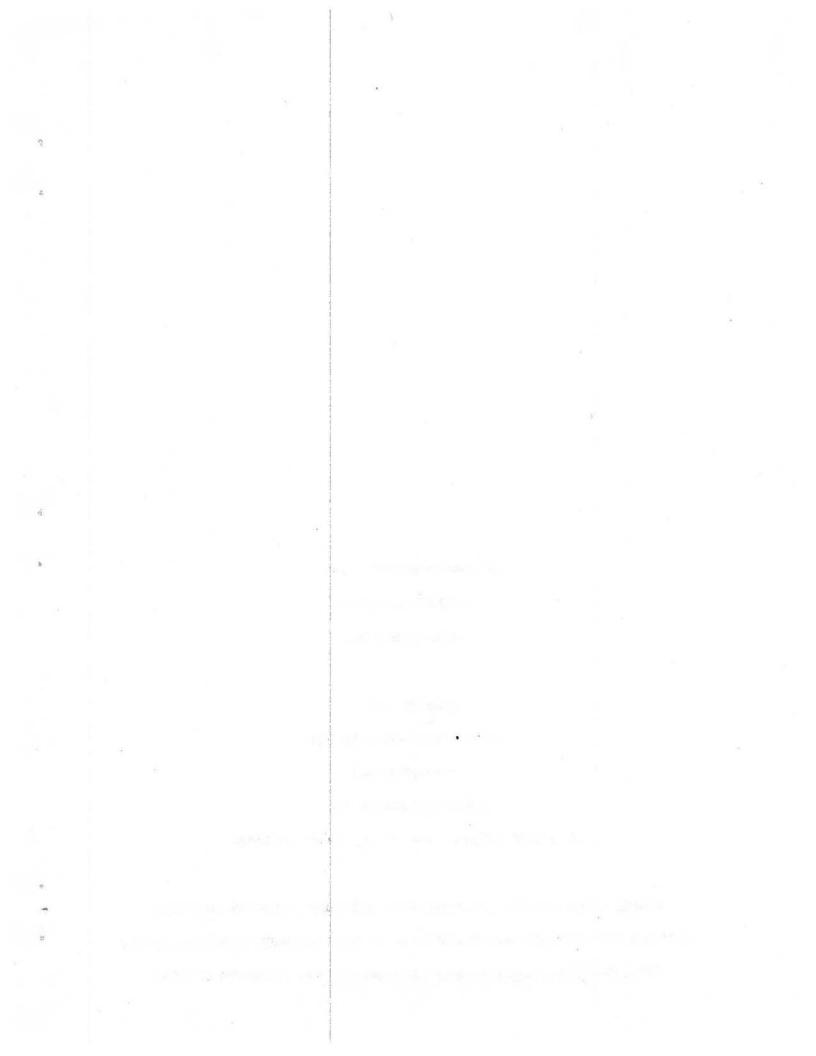
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Executive Summary

Mosquito control districts often use ultra low volume (ULV) applications of insecticides to control adult mosquitoes. Few field studies have tested the effects of these applications on non-target species. We quantified the effect of ULV applications of synergized pyrethrin, permethrin, and malathion on non-target invertebrates and mosquitofish inhabiting seasonal wetlands on the Sutter and Colusa National Wildlife Refuges (NWR)s in central California. Synergized pyrethrin was applied over three irrigated fields at Sutter NWR, and malathion and permethrin were each applied over two fields at Colusa NWR. There were three control fields at each site. We monitored the abundance, biomass, and species composition of aquatic invertebrates before and after treatments, analyzed water samples for pesticide residue, counted flying insects 'knocked down' by the treatments, trapped flying insects (Colusa only), and tracked survival of caged adult and larval mosquitoes and mosquitofish. Survivorship of larval mosquitoes indicated whether pesticides were deposited in the water in quantities lethal to aquatic insects. We also tested the efficacy of pyrethrin against mosquitoes in riparian vegetation.

No reductions were detected in the total abundance or biomass of aquatic macro-invertebrates in treated or control fields in any trial. Aquatic Coleoptera and Ephemeroptera showed lower post-treatment abundances in one treated field each (permethrin and malathion, respectively) but not in the other of the same treatment. Larval mosquitoes showed high survival in all treated and control fields. Mosquitofish died from unrelated causes at Sutter NWR, but all survived at Colusa NWR.

All adult mosquitoes died when caged over fields treated with malathion and permethrin. All survived in control fields, indicating that insecticide did not drift into control areas. Abundances of flying insects decreased in both treated and control fields when insecticides were applied. Flying insect abundance rebounded 24 h later in both sets of fields. Our tests of the efficacy of ULV application of pyrethrin in riparian vegetation showed that few caged adult mosquitoes died when wind speeds were approximately 1 mph, but mortality was evident when wind speed was 8- 10 mph. Higher wind speeds allow better penetration of the vegetation.

The results of our study suggest that ULV applications of these insecticides for the control of adult mosquitoes do not substantially affect the abundance of aquatic macro-invertebrates or fish in treated wetlands. The reasons for this are discussed.

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INTRODUCTION

There is a substantial public interest in keeping wetland habitats healthy and productive because wetlands are vital to many species that are ecologically, economically and aesthetically important, and some of these are declining or endangered. Wetlands, however, also produce mosquitoes and other blood-feeding arthropods, some are pest species and others transmit pathogens causing serious diseases in wildlife, humans and domestic animals (e.g. Eldridge 1989, review, Washino and Dritz 1995). To control pestiferous mosquitoes and reduce the risk of vector-borne disease, publicly-supported mosquito control agencies actively suppress mosquito populations. Mosquito control can be accomplished by reducing or eliminating mosquito habitat (source reduction), killing larval mosquitoes (larviciding) and killing adult mosquitoes (adulticiding). Mosquito control districts choose methods to control mosquitoes in their areas based on environmental impact, feasibility, and economics. We review the major strategies of mosquito control below, before focusing on our experimental study of adulticides.

Source reduction has historically involved draining or impounding wetlands, but these tactics are being modified or phased out because they can be destructive to wetlands and wetland-dependent plant and animal species. Modern source reduction programs include manipulation of wetland hydrology or vegetation to reduce mosquito production while preserving most other wetland species (Provost 1977, Carlson et al. 1991, Batzer and Resh 1992, De Szalay et al. 1996). The feasibility and effectiveness of source reduction is site-specifiq, because not all areas are easily modified and manipulating habitat can sometimes harm sensitive species. Source reduction principles can help guide wetland restoration projects, to minimize mosquito problems in newly

constructed wetlands. Further research is needed to develop environmentally sound source reduction techniques.

Larvicides are insecticides that kill mosquito larvae. Because mosquito larvae are aquatic and occur in delimited habitats, larvicides can kill mosquitoes before they mature and disperse, limiting insecticide applications to aquatic source areas. Most contemporary larvicides break down rapidly. Certain bacterial larvicides and microbial pathogens are highly specific, only affecting mosquitoes and closely related taxa (e.g. Bacillus thuringiensis israelensis, Bacillus sphaericus, Lagenidium giganteum), while others are general insecticides (e.g. temephos, methoprene, golden bear oil) (reviews, Mulla 1994, Washino and Dritz 1995). Larvicides are used successfully in most areas but efforts to control mosquitoes using larvicides sometimes fail. In some cases, mosquito larvae are so abundant that not enough insecticide can be applied so that all get a lethal dose. Some breeding areas are too numerous or inaccessible for inspection and treatment (i.e. treeholes, crab holes, snowmelt holes). Other control failures can result from inadequate inspections or incomplete treatments. Finally, some areas have such extensive mosquito-breeding habitat that larviciding is not feasible logistically and economically. In these cases, mosquito control districts may apply adulticides around human habitation and in areas where mosquitoes swarm and rest.

Adulticides are used to kill adult mosquitoes. To kill flying adult mosquitoes, insecticides are often applied as ultra-low volume aerosol fogs (ULV fogs). Although most modern pesticides applied as ULV fogs show low toxicity to most vertebrates and break down rapidly in the environment (reviews, Mulla 1994, Washino and Dritz 1995), laboratory studies show that they are toxic to many invertebrates and fish. Wildlife refuge managers are concerned that the use of these pesticides might harm refuge ecosystems by reducing the abundance of insects and other invertebrates that are important food for birds and fish, or by directly harming fish. Although birds and other terrestrial vertebrates may consume insects that have been exposed to pesticides, this is not expected be harmful because these compounds are orders of magnitude less toxic to birds and mammals (Hill 1989, Smith 1987).

In the Sacramento Valley and elsewhere, ULV insecticide fogs are one of the mosquito control techniques used by mosquito abatement districts in wetland areas inside and outside of National Wildlife Refuge boundaries. The objective of our study was to quantify the effects of three commonly used insecticides; pyrethrin, permethrin, and malathion, on the abundance and biomass of aquatic invertebrates and fish in seasonally flooded freshwater wetlands in the Central Valley of California. Below we review the role of Central Valley wetlands as wildlife and mosquito habitat, and discuss mosquito-borne diseases and properties of the insecticides under study.

The Central Valley of California is the primary overwintering area for over 60% of the waterfowl in the Pacific flyway (Frayer 1989). Extensive tracts of private and public lands, including National Wildlife Refuges (NWRs), are managed as seasonal wetlands to provide habitat for these birds and other wildlife. Sacramento Valley seasonal wetlands are also important habitat for mosquitoes such as <u>Aedes melanimon</u> Dyar and <u>Culex tarsalis</u> which are significant biting pests and which can vector diseases of humans, livestock, and wildlife.

<u>Ae. melanimon</u> is a facultative multivoltine mosquito that oviposits on dry areas that flood seasonally. Its eggs hatch only during periods of inundation following dry periods, and it can achieve high adult densities within one to three weeks of flooding events. <u>Ae. melanimon</u> is the primary vector and reservoir of California encephalitis (CE) virus. The virus is maintained by transovarial transmission from infected females to their progeny via the egg as well as horizontal transmission to vertebrate hosts by bite (Turrel

et al. 1982). <u>Aedes melanimon</u> on the Colusa NWR were found to have a high biological capacity to transmit arboviruses based on high sustained abundance and survivorship (Jensen and Washino 1991). It is a significant biting pest because of its high abundance and because it readily disperses into population areas and bites humans (Richards 1956).

<u>Culex tarsalis</u>, another mosquito that exploits seasonal wetlands as larval habitat, is the principal vector of western equine encephalomyelitis (WEE) virus (Reeves 1990). <u>Cx. tarsalis</u> lays its eggs on standing water and they develop immediately. Populations of <u>Cx. tarsalis</u> may build in wetlands that remain flooded if natural predators are scarce or are impeded by dense vegetation. Both <u>Cx. tarsalis</u> and <u>Ae. melanimon</u> play an important role in WEE epidemiology in the Sacramento Valley (Hardy 1987). WEE is thought to be sustained in the Sacramento Valley through two interrelated transmission cycles, one involving <u>Cx. tarsalis</u> and wild birds, and a second involving <u>Ae.</u> <u>melanimon</u> and jackrabbits as the primary host (Hardy 1987).

Increased WEE virus activity in the wild bird population is associated with increased vector mosquito abundance (Tsai and Mitchell 1989, Reeves 1990). Mosquito abatement districts reduce the risk of virus transmission to humans and domestic animals by actively suppressing vector mosquito populations. To control adult mosquitoes emerging from seasonal wetlands, synergised pyrethrin (Pyrocide™), permethrin and malathion are applied as ULV fogs. The quantity of material applied for the control of adult mosquitoes is substantially less than the amount applied for the control of agricultural pests. The maximum label rate for ULV malathion application for mosquito control in California is 4 fluid ounces per acre, but in edible crops it can be used at rates up to 16 fl. ounces/ acre. Similarly, for pyrethrin, the label rate for mosquito control is 0.04 ounces/acre, in contrast with up to 16 ounces per acre for

crops. These materials are non-specific insecticides so there is a concern that their application can reduce the abundance and biomass of macro-invertebrates other than mosquitoes, thereby reducing the potential food source for migratory waterfowl and other insectivorous animals.

Pyrethrin is a botanical derivative (from chrysanthemums) that has strong insecticidal properties. Pyrethrin and permethrin are in a chemical class called the 'pyrethroids', all of which have similar chemical structures to pyrethrin. Pyrethroids break down rapidly in sunlight and readily'adsorb to surfaces and particles because they are lipophilic (Coats et al. 1989, Hill 1989), and these features may reduce their availability to organisms in the environment. Because most laboratory tests of toxicity are conducted indoors using clear water, the quantity of pyrethroids to which organisms are exposed may exceed field exposures by orders of magnitude (Hill 1989, Clark et al. 1989, Day 1989). Field tests are therefore necessary to determine the potential impacts of pyrethroids applied over or near wetlands.

Few studies have assessed the impact of these compounds on aquatic invertebrate abundance or biomass. Most published studies are of forestry or agricultural applications, where pyrethroids were applied at higher rates than used for mosquito control. Studies of the effect of drift or runoff of permethrin in silviculture or agriculture have found no effect on aquatic organisms (Frank et al. 1991), transient behavioral changes but no mortality (Werner and Hilgert 1992), or drift and mortality when the permethrin was added directly to the water or deliberately used under conditions that cause heavy deposition (Kreutzweiser and Kingsbury 1987, Sibley et al. 1991, Helson et al. 1993; review: Smith and Stratton 1986).

Malathion is an organophosphate insecticide that has low mammalian toxicity and short persistence in the environment (Mulla et al. 1981, Smith 1987). Effects of

malathion on non-target aquatic organisms are even less well-known than those of pyrethroids. When applied directly into the water, it has been shown to decrease amphipod populations (Crane et al. 1995) and it affects fish under laboratory conditions (Beyers et al. 1994, Shao-nan and De-fang 1996). The non-target effects of ULV application for the control of adult mosquitoes are not known.

The objectives of the studies reported herein were to assess the effect of ULV application of these insecticides on: 1. the abundance and biomass of aquatic macroinvertebrates, 2. the abundance of flying insects in seasonal wetlands, 3. the survivorship of mosquitofish, <u>Gambusia affinis</u>. A further goal was to assess the field efficacy of ULV application of pyrethrin to control adult <u>Ae</u>. <u>melanimon</u> in riparian areas, where dense vegetation could impede spray drift.

Our studies were conducted in seasonal wetlands on the Colusa and Sutter NWRs in the Sacramento Valley of California, and riparian woodlands at Sutter NWR. Seasonal wetlands are intentionally flooded in late summer and fall to provide habitat for overwintering migratory waterfowl. Fields remain covered with standing water during winter and are drained in spring. These fields may produce an initial hatch of <u>Ae</u>. <u>melanimon</u>, and some may continue to produce other mosquitoes such as <u>Cx. tarsalis</u> until low winter temperatures curtail mosquito development. Some fields, including our study sites on the Sutter NWR, are briefly flooded in late spring to irrigate desirable vegetation and/or to control undesirable vegetation; these irrigations can produce <u>Ae</u>. <u>melanimon</u> , but they typically do not last long enough to generate problems with <u>Cx.</u> <u>tarsalis</u> and other mosquitoes. We studied the non-target effects of ULV pyrethrin during a late spring irrigation on Sutter NWR, and the effects ULV of malathion and permethrin during fall flooding on Colusa NWR. We provide methods and results for the Sutter and Colusa studies separately, below.

METHODS: SUTTER PYRETHRIN STUDY

We studied the effect of ultra low volume (ULV) adulticide application of synergised pyrethrin (Pyrocide[™] 5%) on aquatic macro-invertebrates and adult mosquitoes during irrigations on the Sutter National Wildlife Refuge (NWR) in June 1996. Vegetation in the Sutter NWR irrigated seasonal wetlands included bermudagrass (Cynodon dactylon), jointgrass (Paspalum distichum), watergrass (Echinochloa sp.), smartweeds (Polygonum sp), and sprangletop (Leptochloa sp). The riparian areas were comprised of multistoried trees, shrubs, and ground vegetation, including cottonwood (Populus fremontii), willow (Salix sp.), buttonbush (Cephalanthus sp)., Blackberry (Rubus discolor), and a variety of annual and perennial grasses.

We compared macro-invertebrate abundance and biomass in three fields to which pyrethrin was applied and three adjacent control fields (Figure 1). Treated fields were paired with proximate control fields located upwind of treated fields to prevent pesticide drift over control areas. Flooding was completed three to five days before pyrethrin application, at which time sampling commenced. Macro-invertebrate populations were sampled daily using aquatic sweep nets and standard mosquito dippers. Sweep net collections were made using 1 mm mesh standard d-ring nets along four transects in each field. Each transect consisted of 30 standardized sweeps 1 m apart along the substrate. Sampling continued until 7 days post application, at which time the fields were drained. We also sampled mosquito larvae and other surface invertebrates along the edge of each field in four 25 dip transects daily.

We measured the abundance, diversity and biomass of organisms in sweep net collections from treatment and control fields by sorting, counting and identifying organisms to taxa. To determine biomass, we pooled the collected organisms, dried them for 72 h in a drying oven and weighed them. Data were analyzed by linear

regression of time-series to detect any decreases in total abundance or biomass of aquatic invertebrates after insecticide application, and by one-way analysis of variance (ANOVA) of the mean abundance of invertebrates averaged over the sample dates before versus after the insecticide application. Invertebrate dynamics in control and treated areas were compared, because invertebrate numbers may change over time via natural causes that include colonization, breeding, metamorphosis, competition, and predation. Our purpose was to identify changes that only occurred in treated areas.

We also performed exploratory, separate ANOVAs for each of the dominant taxa collected. These are classed as exploratory because analyzing several individual taxa from the same site is equivalent to using multiple response variables from the same experimental treatment, which renders the usual 0.05% significance level inaccurate (Sokal and Rohlf 1995). The chance of finding an erroneously significant result increases as more tests are performed. The 'P' value accepted as significant can be adjusted downward to compensate for multiple tests, however this statistically conservative approach can result in very little power to detect real differences. This is perhaps undesirable in a study designed to discover potential threats to wildlife resources. Low P values that are non-significant when adjusted for multiple comparisons would ideally be used to identify areas for future research.

To determine the effect of ULV application of pyrethrin on aquatic organisms known to be highly susceptible to pyrethrin, we exposed <u>Ae</u>. <u>melanimon</u> and <u>Cx</u>. <u>tarsalis</u> mosquito larvae to the ULV pyrethrin application by placing the larvae in floating predator exclusion cages in both treatment and control fields and comparing survivorship between fields. There were two cages of 25 larvae per species per field. <u>Ae. melanimon</u> were field-collected and <u>Cx. tarsalis</u> were from an insecticide-susceptible laboratory colony. The predator exclusion cages had screens allowing water exchange

between the cage interior and the field. Cage tops were removed before pyrethrin application to expose larvae to the insecticide, and were replaced shortly thereafter. Cages were monitored daily until day 7, at which time nearly all larvae had died or pupated. Data were analyzed by analysis of variance (ANOVA), and proportions of mosquitoes surviving were arcsin-square root transformed before analysis to meet the assumptions of ANOVA. Data points were the averaged proportion surviving in the two buckets per species per impoundment; averaging these values avoided pseudoreplication problems in data analysis (Hurlbert 1984). We report larval mosquito survival 24 and 168 hours post-spray. The pesticides should kill mosquitoes within a few hours, so the 24 h mortality is most likely to reflect pesticide activity rather than other causes of mortality. However, we also report survival at 7 days, in case the pesticides had unexpected delayed effects.

We also attempted to assess the effect of ULV pyrethrin application on the survivorship of the mosquito fish, <u>Gambusia affinis</u>. Five fish were placed in each of two predator exclusion cages in each field. Cages were exposed to the insecticide in the same manner as the sentinel mosquito larvae.

To assess the effect of ULV pyrethrin application on flying insects other than mosquitoes, we set out 1m² plastic knockdown boxes at distances of 1, 5 and 10 m perpendicular to the spray route in each treatment and control impoundment just before pyrethrin was applied. These boxes were collected 20 minutes later and dead insects in the boxes were identified and counted.

Pre- and post-application surface water samples were collected from each treatment and control field. Each sample was a combination of three 1/3 liter subsamples of surface water that were collected at approximately 5, 10, and 15 m from the spray route. Subsamples were collected by drawing water from the top 2-4 cm layer

of water into inert sample bottles. Samples were refrigerated until analysis, and were analyzed within 24 h of collection for the presence of pyrethrin by the toxicology section of the California Veterinary Diagnostic Laboratory System - Davis.

The efficacy of ULV pyrethrin application in controlling adult <u>Ae</u>. <u>melanimon</u> mosquitoes in riparian vegetation was tested on Sutter NWR in June and September . 1996. Caged sentinel adult female <u>Ae</u>. <u>melanimon</u> mosquitoes were set out at three locations along the spray route and three control sites at distances of 1, 5, 10 and 15m into woody riparian vegetation. In June, single cages were set out at 1 m in height. In September, we set out 2 cages at each distance; at 1 m in height and a second at ground level. Each cage contained 18-28 mosquitoes.

Pyrethrin was applied across the three treatment impoundments and along the edge of a strip of riparian vegetation by a Sutter-Yuba Mosquito and Vector Control District operator using a truck mounted Becomist ULV spray unit.

RESULTS: SUTTER PYRETHRIN STUDY

The application rate for pyrethrin was 4 oz/min (based on a calibration of the spray machinery) at 10 mph truck speed. Droplet size ranged from 1 - 31 microns with a mean of 14.3 microns in diameter. The application commenced at 20:05 on June 11, 1996 and was completed by 21:26. Air temperature decreasing from 28.6° to 25.2° C, and there was an inversion of $1.3-2.4^{\circ}$ C.

Survivorship of <u>Ae</u>. <u>melanimon</u> and <u>Cx</u>. <u>tarsalis</u> larvae in the predator exclusion cages was high (in general over 70% at day 7, Table 1) with no significant difference in survivorship detected for either species between treatment and control fields. Field collected <u>Ae</u>. <u>melanimon</u> larvae, however, survived better in field cages than colony <u>Cx</u>. <u>tarsalis</u> (Table 2). We were unable to assess the effect of pyrethrin on sentinel <u>Gambusia affinis</u> survivorship because nearly all fish in both treatment and control fields died within 12 h. The fish were stressed when transported to the study site and many were dead or moribund on arrival. One female in one treatment field, however, gave birth before the site was treated and none of her progeny died, suggesting that ULV pyrethrin application does not kill newborn mosquito fish.

The abundance of aquatic macro-invertebrates in individual treatment and control fields are presented in Figures 2 - 7. The abundance and diversity of aquatic macro-invertebrates in the newly flooded in fields was initially very low. Only aquatic beetles (Dytiscidae and Hydrophilidae), snails and <u>Ae</u>. <u>melanimon</u> larvae were abundant in the aquatic sweep net collections. <u>Aedes melanimon</u> larvae disappeared shortly before the spray date, when the cohort completed larval development, pupated and emerged as adults. This emergence was similar to emergences that could have resulted in adulticide application under non-experimental conditions. Mayfly nymphs (Ephemeroptera) were abundant in several fields by the end of the study period but were excluded from analysis because they were absent when pyrethrin was applied.

No significant decreases or significant negative trends were observed in numbers of snails, dytiscid adults or larvae, and hydrophylid larvae collected each day in any treated impoundments when examined using analysis of variance (ANOVA) and linear regression of time series for numbers of snails, dytiscid and hydrophilid beetle larvae and dytiscid adults collected each day, respectively. Analysis of variance tables comparing the mean numbers of snails, dytiscid beetle adults and hydrophilid beetle larvae collected each day before and after pyrethrin application are presented in Tables 3 - 5.

There was, however, a trend toward increasing abundance in both treated and control impoundments, consistent with colonization and subsequent breeding in the newly flooded impoundments. The abundance patterns for the total number of organisms collected each day in the sweep net collections are presented in Figures 8 - 10. These exhibit a high degree of concordance in population fluctuations between paired treatment and control fields, suggesting that pyrethrin application did not decrease macro-invertebrate abundance.

The dried biomass of aquatic macro-invertebrates collected each day in paired treatment and control fields are presented in Figures 11 + 13. Analysis of variance of dried biomass before and after pyrethrin application (Table 6) found significant increases in biomass in treatment and control fields T12 C1 and T12 C3, respectively, but no significant decreases for any treatment or control field. Mean daily biomass did, however, differ significantly by field (F = 10.29, d. f. = 5, 56, p < 0.001). Regression analysis gave similar results: dried biomass increased significantly over time in treatment fields T12 C1, T19, and in control field T12C3. (T12C1: X = 0.357 + 0.0661Y, T = 3.77, p < 0.004; T19:X = 2.44 + 0.21Y, T = 2.60, p < 0.03; T12C3: X = 0.845 + 0.310Y, T = 9.00, P < 0.001) with no significant changes in dried biomass observed over time in the remaining fields.

Too few macro-invertebrates were collected in dipping transects at the edge of the treatment and control fields for analysis.

Only 2 flying insects, a chironomid midge and a tachinid fly, were collected from the 9 knockdown boxes in the treatment fields and a single male <u>Ae</u>. <u>melanimon</u> mosquito was collected from the boxes in a control field. This suggests that flying insect densities were too low to be effectively sampled using the 1 m² knockdown boxes.

Pyrethrin was not detected at < 0.02 ppm detection limits in pre or post treatment surface water samples collected from each treatment and control field on June 11.

Almost all adult <u>Ae</u>. <u>melanimon</u> mosquitoes in sentinel cages in the riparian vegetation survived on 11 June, indicating that this pyrethrin application was not efficacious. Wind speed was very low (< 1 mph on site) and the spray route was almost parallel to the prevailing wind direction so it was likely that low drift rates resulted in the ULV cloud failing to penetrate the riparian vegetation.

In contrast, efficacy was high when this study was repeated in September. Conditions were substantially different at this time with wind speed of 9 - 10 mph and wind direction was perpendicular to the vegetation edge. In the three treatment transects, overall mortality ranged from 88.1% to 100% compared with 0 - 1.1% mortality in control transects. All insecticide exposed mosquitoes in cages at 1 m in height died as did 196/197 in cages at ground level less than 15m from the spray route. Mortality was, however, lower in furthermost cages at ground level. At 15m, survivorship was 0%, 52% and 83% in the three transects, suggesting that pyrethrin has a limited capacity to penetrate riparian vegetation at ground level.

METHODS: COLUSA PERMETHRIN AND MALATHION STUDY

We studied the non-target effects of ULV applications of malathion and permethrin during the fall flooding period in a series of paired fields on the Colusa NWR (Fig. 14), using methods similar to those in the pyrethrin study. These fields were broadly similar in vegetation to those at Sutter NWR, although they had more patches of emergent vegetation (primarily bulrushes and cattails). Plants species included bulrushes (Scirpus acutus, S. maritimus, S. tuberosus, S. fluviatilus), cattail (Typha sp.), swamp timothy (Crypsis sp.), and cocklebur (Xanthium sp). Two treatment fields were established for each insecticide and these were paired with concurrently flooded control fields upwind of the treated fields. Because of the low diversity and abundance of aquatic macro-invertebrates in newly flooded impoundments during the Sutter NWR study, we delayed insecticide application until all fields had been fully flooded for >2 weeks. This allowed additional time for immigration and reproduction to increase the diversity and abundance of aquatic macro-invertebrates. Although this delayed treatment past the emergence and dispersal of <u>Ae</u>. <u>melanimon</u> from the study area, it provided a larger range and greater abundance of non-target organisms, allowing a more extensive test of non-target effects of these insecticides. Adulticides are often applied over these fields this late after flooding because of later breeding by <u>Cx. tarsalis</u> and because mosquitoes from surrounding ricelands rest and swarm in the area.

Aquatic sweep nets and standard mosquito dippers were again used to sample the aquatic fauna before and after treatment. Two sweep net transects of 10 sweeps each, and a 25 dip collection along a transect along the plant/surface interface were made in each impoundment 5, 4, and 1 day(s) pre-application and on post-treatment days 1-3, 5, 7 and 14. Fewer sweeps were needed than in Sutter NWR because of the greater abundance of invertebrates. Invertebrates were preserved in 70% ethanol for subsequent identification to taxon, enumeration, and biomass measurement.

We compared the survivorship of mosquitofish and a strain of insecticidesusceptible <u>Cx</u>. <u>tarsalis</u> larvae in predator exclusion cages in treated and control fields. We used a laboratory strain of mosquitoes because local <u>Cx</u>. <u>tarsalis</u> populations show some resistance to malathion and wild <u>Ae</u>. <u>melanimon</u> larvae were unavailable when the study was conducted. Two cages of 25 mosquito larvae each and two cages containing four mosquitofish were placed in each field prior to insecticide application. Predator exclusion cage lids were removed during insecticide application and replaced shortly

thereafter. Plastic knockdown boxes were placed in all impoundments. Transects of sentinel cages containing 20-31 adult female <u>Ae</u>. <u>melanimon</u> mosquitoes were placed in open areas 10, 15 and 20 meters from the edge in each field in treated impoundments.

Control impoundments were upwind but adjacent to spray routes. We placed adult sentinel cages in these impoundments next to the spray routes to detect possible contamination that could result from ULV drift in an unexpected direction. Adult sentinel cages were placed at 1, 5, and 10 meters into control impoundments.

We used Center for Disease Control mosquito traps emitting ultraviolet light (UV CDC) to sample the composition and abundance of flying insects over each impoundment. One trap was placed in each impoundment 25 m from the edge, and collections were made during three nights before insecticide application (24-25, 25-26, and 26-27 September), on the night insecticide was applied (30 Sept - 1 Oct.), and on two subsequent nights (1 - 2, and 2 - 3 October).

We collected pre- and post-spray samples of surface water from all impoundments for insecticide analysis using methods identical to those for the pyrethrin study (above). Insecticides were applied using the same equipment as the previous study.

RESULTS: COLUSA PERMETHRIN AND MALATHION STUDY

Malathion and permethrin were applied at rates of 8 oz/min and 5 oz/min, respectively, at speeds of 10 mph between 19:16 and 19:37 on September 30 1996. Droplet size was in the same range as in the previous study. Air temperatures decreased from 22.7 to 21.3° C with an inversion of 0.4 to 0.7° C. Wind originated from the east/southeast at speeds of 2 - 3 mph during insecticide application, but later rose to greater than 10 mph within an hour post-application. Permethrin was not detected in any water sample but malathion was detected post application in samples from each of the malathion-treated impoundments at 0.006 ppm. Malathion was not detected in any pre-application samples or in post treatment samples from other impoundments.

All caged adult mosquitoes placed in permethrin and malathion treated impoundments died within 24 h. No mosquitoes in cages in the adjacent control impoundments died, indicating that control impoundments were not contaminated by insecticide drift.

Survivorship of sentinel <u>Cx</u>. <u>tarsalis</u> mosquito larvae did not differ significantly between control fields and those treated with either permethrin or malathion (Table 7; ANOVA on arcsin-square root transformed proportion surviving 24 h: permethrin vs. control: $F_{1,3} = 2.637$, P = 0.203, malathion vs. control: $F_{1,3} = 0.245$, P = 0.655; proportion surviving until day 7, permethrin vs. control: $F_{1,3} = 2.420$, P = 0.21, malathion vs. control: $F_{1,3} = 0.312$, P = 0.61).

The diversity and abundance of organisms in these fields was greater than in the Sutter NWR study. Midge (Chironomidae) and beetle (Coleoptera) larvae, dragonfly (Anisoptera), damselfly (Zygoptera), and mayfly (Ephemeroptera) nymphs, and water boatmen (Corixidae), back swimmers (Notonectidae) and snails were abundant in these impoundments. Mosquito abundance was, however, very low; only a few <u>Culex tarsalis</u> and <u>Anopheles freeborni</u> larvae were collected.

The number of organisms of different taxa collected in sweep net and dipper collections each day are presented in Figs. 15 - 21 and 22 -28. Comparison of time series of the total number of organisms of collected each day in the daily sweep net and dipper collections in the paired treatment and control fields are presented in Figs. 29 - 32 and 33 - 36, respectively. Treated fields did not show decreases in the abundance of

aquatic invertebrates after the spray relative to control cells, and most fields showed a trend toward increased abundance. The abundance of various taxa in matched treated and control cells fluctuated in parallel throughout the sampling period. Time series analysis (linear regression) of sweep net samples showed no significant decreases over time in numbers of Chironomidae, Coleoptera, Anisoptera, Zygoptera, Ephemeroptera, Corixidae, Notonectidae and snails. Similarly, with few exceptions, we did not observe significant differences in mean numbers of organisms in sweep nets collected each day pre- and post treatment using analysis of variance (Tables 8 - 15). However, there was a significant decrease in Coleoptera in one of the permethrin treated fields (Table 9), and a decrease in Ephemeroptera in one of the malathion treated fields (Table 9), and a decrease in Ephemeroptera abundances did not decrease in the other malathion and permethrin treated fields, but one control field also showed a significant decrease in Ephemeroptera.

No significant trends in dried biomass over time were detected in any field during the Colusa NWR study (Figs. 37 - 40). Similarly, we did not detect significant changes in mean dried biomass of aquatic macro-invertebrates from sweep net collections before and after insecticide application (Table 16). Mean daily biomass did, however, differ significantly by field (F = 9.8, d.f. = 6,56, p< 0.001).

Similarly, we did not detect significant decreases in mean numbers of individuals collected each day in the dipper collections for Ephemeroptera, Anisoptera, Chironomidae, Zygoptera, Coleoptera and Corixidae (Tables 17 - 22).

Dead insects were present in over half of the knockdown boxes, however numbers were low and variable and there were no significant differences between treatments. Mean numbers of insects collected from each box were 3.8, 13.3 and 6.5 for

control, permethrin and malathion treated fields, respectively. Seventy-five percent of the insects collected were Chironomidae, and the rest of the collection was comprised of other Diptera and one odonate (Table 23).

All mosquito fish survived in every impoundment, indicating that mosquito fish were not killed by ULV application of permethrin or malathion.

Field treatment had no significant effect on the abundance of flying insects collected in CDC light traps, however a repeated-measure ANOVA showed that abundances differed between dates (Table 24). In all fields, there was a marked drop in the abundance of insects on the night of pesticide application (Figures 41-44). Abundances rebounded and were similar to the initial population levels during the next sampling dates. Insects collected included Diptera (Chironomidae, Culicidae, Tipulidae and others), Lepidoptera, Coleoptera, and a few Hemiptera. Figure 45 shows the proportion of different taxonomic groups collected in light traps in each field averaged across three days before insecticide application and three days afterward.

High wind speeds can reduce light trap catches (see discussion), and wind velocity was higher on the spray night. Mean wind speeds at nearby Beale Air Force Base in Marysville, CA were recorded as follows by NOAA National Weather Service: Sept. 24, 25 and 26, were 3.0, 1.9 and 2.9 knots, respectively; Oct. 1 and 2 were 6.3 and 4.3 knots.

DISCUSSION

Total numbers of aquatic non-target organisms fluctuated in parallel in pyrethrintreated and control fields in the Sutter NWR. This high degree of concordance, and the lack of a decrease in numbers after insecticide application indicates that pyrethrin did not have any detectable effect on the abundance of non-targets. This conclusion is strengthened by high survivorship of sentinel mosquito larvae in treated fields. Since pyrethrin is a general insecticide, the lack of effect of pyrethrin on aquatic organisms probably results from low exposure of the organisms. Pyrethrin was not detectable in surface water samples analyzed within 24 h of the spray. Low exposure could result from low deposition into the water, rapid breakdown of the insecticide, and adsorption of the insecticide to particulates in the water and substrate. ULV fogs ordinarily minimize deposition of insecticides because of the low volume of the spray and its distribution over a wide area (Lofgren 1970). Pyrethroids like pyrethrin typically show low persistence in the environment because of rapid breakdown or adsorption to particles (e.g. Coats et al. 1989, Hill 1989). We did not differentiate between these mechanisms.

The efficacy of ULV pyrethrin for mosquito control in riparian areas depended on meteorological conditions. The first application was performed when wind direction was oblique to riparian vegetation and wind speeds were low, and it failed to control mosquitoes in riparian areas. Pyrethrin killed sentinel mosquitoes in the riparian areas during the second trial when wind speeds were higher and the direction was perpendicular to the vegetation, however mortality was lower further from the spray route. The wind direction and velocity likely facilitated penetration of riparian vegetation by the insecticide particles resulting in mosquito mortality. Mosquito control agencies could conserve labor and maximize effective use of insecticides by applying ULV materials in vegetated areas only when conditions allow the material to effectively penetrate the vegetation. To accomplish this most effectively, wind speed should be measured within the vegetation where possible, to ensure that speeds are high enough to carry material through the vegetation.

During the pyrethrin trial in newly irrigated fields at Sutter NWR, many aquatic macro-invertebrates were absent or present in very low densities. The floodwater mosquito <u>Ae</u>. <u>melanimon</u>, however, is sometimes very abundant in newly flooded areas,

because eggs containing fully formed first instar larvae are often present before the site floods, and these hatch shortly after they are immersed. Because of <u>Ae. melanimon's</u> rapid emergence, adulticidal treatment can be applied before many other non-target aquatic organisms are present, precluding their exposure to the insecticide. The timing of our application of pyrethrin coincided with the normal timing of control of <u>Ae.</u> <u>melanimon</u> in this area (within 1-3 days of adult emergence). Ephemeroptera, Anisoptera, and Zygoptera were absent until after the mosquitoes emerged and pyrethrin was applied, and chironomid midges did not colonize these fields during the short flooding cycle.

When impoundments remain flooded, however, other mosquitoes and non-target organisms colonize. Whether these mosquitoes require treatment depends on their numbers and on virus activity in the area. Therefore, we conducted our second study in impoundments that remained flooded for some time because mosquito control often occurs when a more diverse non-target community is present. In the Sacramento Refuges, for example, mosquito abatement districts typically apply adulticides early in the fall flooding period when water temperatures are high enough to allow mosquitoes to breed.

Results of the applications of malathion and permethrin were very similar to those for pyrethrin. There were no detectable decreases in the total abundance or biomass of aquatic invertebrates after insecticide application. These parameters fluctuated in parallel in paired treatment and control fields. However, exploratory data analysis indicated decreases in the abundance of Coleoptera and Ephemeroptera in single impoundments treated with malathion. More research would be necessary to confirm effects of malathion on Coleoptera and Ephemeroptera because in both cases, the effect was not consistent between replicates. The decrease in Coleoptera would not

be significant if the probability value were corrected for multiple comparisons. For Ephemeroptera, numbers were very low and thus potentially unreliable in the impoundment where the effect was detected. There were fewer than four mayflies in twenty sweeps before treatment, and the difference between pre- and post-treatment was less than three mayflies. A control field also showed a decrease in Ephemeroptera.

There were no differences in larval mosquito and mosquitofish survival in sentinel cages in treated versus control impoundments. In contrast, there was 100% mortality of adult mosquitoes caged immediately over the fields, indicating that effective mosquito control can be accomplished without deposition of insecticide into adjacent wetlands at levels that cause mortality in fish and insects.

No permethrin was detected in water samples, however malathion was present at 0.006 ppm in post-treatment samples from fields treated with malathion. To put this concentration in perspective, it is over an order of magnitude below the LC50 dosage producing acute toxicity in fish. Shao-nan and De-fang (1996) tested five species of fish representing five families, and found an LC50 of 0.25 ppm for the most sensitive species, rainbow trout. Mosquitofish were the second-most sensitive species, with an LC50 of 0.7 ppm.

Prior to this study, Steinke (unpublished data) estimated the expected deposition of malathion into seasonal wetlands at the Colusa NWR by measuring the number and size of pesticide droplets deposited on mylar films at the water surface. He found an expected concentration of 0.8 ppb malathion if water was 0.6 m deep, when malathion applied at 5 oz/min at 10 mph. We found higher levels of the pesticide (6 ppb), possibly because our methods differed. We analyzed surface water collected immediately after the spray. The contaminated surface water may not have mixed thoroughly with the rest of the water column before we took samples.

The knockdown box collections indicated that ULV adulticide fogs killed some flying insects and light trap catches of flying insects also decreased on the night of pesticide application. The light trap results, however, are difficult to interpret because trap catches were low in both treated and control sites. Although the light traps were far enough apart so that they should not have drawn insects from other fields, it is possible that the insects naturally move between fields at high enough rates so that mortality in one area could decrease abundance in adjacent areas through lower dispersal into those areas. However, wind speeds rose just after pesticides were applied, and high wind speeds can decrease light trap catches (Harling 1968, Mizutani 1984, McGeachie 1989). This could also have produced the concordant decrease in light trap catches in treated and control areas. The chironomids and other small Diptera constituting most of the UV light trap collections are weak fliers and will reduce flight activity under windy conditions. Without a difference between treated and control fields, we cannot tell whether low trap catches resulted from insecticide activity or meteorological changes. Regardless of the cause, flying insect abundance recovered by the next evening and remained high during the sampling period. Thus, any loss of insect prev for wildlife was temporary. Although the light trap data is equivocal, adulticide application probably killed a significant number of flying insects as indicated by the greater number of dead insects in knockdown boxes in treated fields and the 100% mortality of sentinel adult mosquitoes.

Neither study detected large or enduring losses of non-target invertebrates caused by use of ULV insecticides. The only consistent, significant changes in the abundance and biomass of aquatic invertebrates were increases in some groups, which is consistent with continued colonization and growth in the impoundments. The lack of detectable mortality in sentinel mosquito larvae and fish caged at the water/air interface

was especially compelling because insecticide concentrations were likely to be highest at the surface. We stress again that larval mosquitoes were 'indicator organisms' for possible effects of the pesticides on insects in the water, and that the insecticides were applied to kill adult rather than larval mosquitoes.

Although pyrethrin, permethrin, and malathion are nonspecific insecticides used to control a variety of insect species, low application rates and the small particle size produced by ULV equipment may have minimized deposition into the water, thereby limiting the exposure of aquatic non-target organisms. Our failure to detect permethrin and pyrethrin, and the low malathion concentrations detected in post treatment water samples indicate that a very small quantity of insecticide was deposited in the treatment fields. These results are consistent with the low deposition rates observed in other studies (e.g. Tietze et al. 1994, Knepper et al. 1996 and references therein). ULV spray equipment produces very small droplets (less than 60 microns in diameter) that tend to remain suspended in air and drift long distances (Lofgren 1970).

Data from the caged adult mosquitoes and possibly the light traps provided evidence that the insecticides caused mortality of flying insects, which is not surprising since they are general insecticides. However, numbers of flying insects rebounded within 24 hours of insecticide application. Risks to wildlife resources may be limited by the timing of ULV adulticide application. These materials are usually applied after sunset, during the period of maximum mosquito flight activity. Daylight flying insects are often inactive after dusk and rest where they are less likely to contact insecticide droplets and be killed. Other crepuscular and nocturnal flying insects such as chironomid midges are active at dusk, and their populations may be temporarily reduced by insecticide application. Loss of these nocturnal flying insects could affect some

wildlife, such as bats, however our study shows that such loss is of short duration and similar to decreases caused by natural phenomena, such as wind or rain.

It is possible that wildlife will consume some contaminated insects, however the opportunity for them to do so is limited because the insecticides feature quick 'knockdown', and many predators eat only living prey. Insects contacted by ULV pyrethroids and malathion should not pose a threat to wildlife because these compounds have low toxicity to mammals and birds. Hill (1989) provides data showing that pyrethroids are relatively non-toxic to mammals and birds. Similarly, Smith (1987) reviewed the toxicity of malathion to animals, and found that LD50's of dietary malathion were well above 100 mg/kg for dogs, rats, mice, quail, mallards, starlings, larks, pheasants, and blackbirds (ranging up to 1,485 mg/kg for blackbirds). Two studies of breeding birds have shown no effect of ULV malathion applications on the fledging success and size of nestling birds (blue tits, Pascual 1994, sage thrashers and Brewer's sparrows, Howe et al. 1996). Malathion was applied during the day in both studies (on two occasions in Howe et al.), at two or more times the label rate for mosquito control in California.

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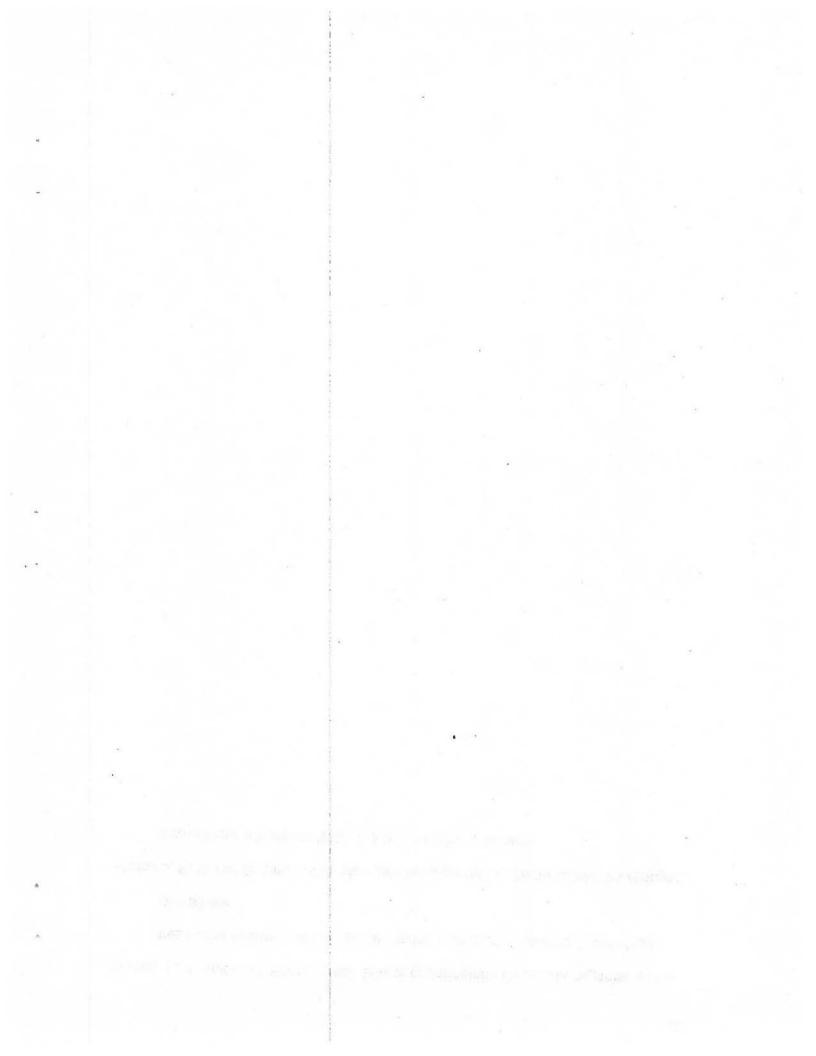


Table 1. Survivorship of <u>Aedes melanimon</u> and <u>Culex tarsalis</u> mosquito larvae exposed to ultra-low volume adulticide application of synergised pyrethrin (Pyrocide[™]) and in control fields on the Sutter National Wildlife Refuge, Sutter Co., California, June 1996.

2			
Species	Treatment	Field	Survivorship
	s remain at an		24 h 168 h
	ng en littlegt re		
Aedes melanimon	Pyrethrin	T2-1	94% 94%
Aedes melanimon	Pyrethrin	T2-1	80% 67%
Culex tarsalis	Pyrethrin	T2-1	100% 85%
Culex tarsalis	Pyrethrin	T2-1	78% 65%
Aedes melanimon	Control	T2-3	93% 77%
Aedes melanimon	Control	T2-3	97% 78%
Culex tarsalis	Control	T2-3	89% 79%
Culex tarsalis	Control	T2-3	82% 68%
Aedes melanimon	Pyrethrin	T12-1	97% 93%
Aedes melanimon	Pyrethrin	T12-1	97% 84%
Culex tarsalis	Pyrethrin	T12-1	100% 85%
Culex tarsalis	Pyrethrin	T12-1	75% 63%
Aedes melanimon	Control	T12-3	100% 77%
Aedes melanimon	Control	T12-3	83% 73%
Culex tarsalis	Control	T12-3	87% 65%
Culex tarsalis	Control	T12-3	89% 74%
Aedes melanimon	Pyrethrin	T20	100% 100%
Aedes melanimon	Pyrethrin	T20	93% 93%
Culex tarsalis	Pyrethrin	T20	83% 64%
Culex tarsalis	Pyrethrin	T20	86% 75%
Aedes melanimon	Control	T19	100% 97%
Aedes melanimon	Control	T19	100% 82%
Culex tarsalis	Control	T19	94% 78%
Culex tarsalis	Control	T19	74% 63%

Table 2. Multivariate analysis of variance of arcsin-square root transformed proportions of sentinel <u>Culex tarsalis</u> and <u>Aedes melanimon</u> mosquito larvae surviving in impoundments beneath a ULV application of pyrethrin versus controls, at 24 or 168 h post-treatment. Data points were the averaged proportions of larvae surviving in two sentinel buckets per species per impoundment. The only difference significant at P < 0.05 was that the laboratory strain of <u>Cx. tarsalis</u> survived less well than field-collected <u>Ae. melanimon</u>. Source is the source of variation, df = degrees of freedom, F = F statistic, p = probability, .

24 hours:

df	F	p
1	7.661	0.024
1	0.703	0.426
1	1.236	0.299
8		/
	1 1 1	1 7.661 1 0.703 1 1.236

168 hours:

Source	df	F	р
Mosquito species	1	8.289	0.021
Treatment	1	0.191	0.674
Species X treatment	1	0.000	0.997
Error	8		

Table 3. One way analysis of variance of the mean number of snails collected each day pre- and post application of synergised pyrethrin in treatment and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA in June 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р	•
2-1	Pyrethrin	73.4 ± 13.4	87.3 ± 24.6	0.22	1, 9	0.651	•
2-3	Control	433 ± 274	301.5 ± 82.7	0.37	1, 7	0.563	
12-1	Pyrethrin	17.8 ± 5.8	32.8 ± 5.8	3.27	1, 9	0.104	
12-3	Control	83.3 ± 44.5	206.3 ± 23.6	7.41	1, 7	0.030	
19	Pyrethrin	258.4 ± 39.9	515 ± 39.9	1.86	1, 9	0.206	
20	Control	1095 ± 269	391 ± 186	4.90	1, 9	0.054	
		<u></u>					

Table 4. One way analysis of variance of the mean number of predaceous diving beetle (Dytiscidae) adults collected daily pre- and post application of synergised pyrethrin in treatment and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA in June 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р	
2-1	Pyrethrin	0.60 ± 0.60	0.83 ± 0.65	0.07	1, 9	0.802	•
2-3	Control	0±0	1.00 ± 0.44	2.33	1,7	0.170	
12-1	Pyrethrin	0.60 ± 0.24	3.33 ± 1.45	2.84	1, 9	0.126	
12-3	Control	4.67 ± 0.67	4.33 ± 1.38	0.03	1, 7	0.877	
19	Pyrethrin	15.40 ± 4.89	23.50 ± 3.66	1.83	1, 9	0.209	
20	Control	2.00 ± 1.10	3.17 ± 0.60	0.96	1, 9	0.353	

Table 5. One way analysis of variance of the mean number of diving scavenger beetle (Hydrophilidae) larvae collected each day pre- and post application of synergised pyrethrin in treatment and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA in June 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р
2-1	Pyrethrin	12.80 ± 2.85	28.67 ± 5.08	6.60	1, 9	0.030
2-3	Control	11.67 ± 3.93	35.83 ± 4.30	12.63	1, 7	0.009
12-1	Pyrethrin	7.40 ± 3.67	'68.00 ± 9.15	32.41	1, 9	0.001
12-3	Control	1.67 ± 1.20	89.8 ± 19.8	9.27	1, 7	0.019
19	Pyrethrin	19.40 ± 3.08	33.17 ± 5.91	3.76	1, 9	0.084
20	Control	26.4 ± 13.6	71.50 ± 7.88	9.01	1, 9	0.015
				1		

Table 6. One way analysis of variance of the mean dried biomass (in grams) of aquatic macro-invertebrates collected each day pre- and post application of synergised pyrethrin in treatment and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA in June 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р	
2-1	Pyrethrin	0.30 ± 0.01	0.38 ± 0.05	0.46	1, 9	0.513	
2-3	Control	1.20 ± 0.54	0.84 ± 0.11	0.85	1, 7	0.387	
12-1	Pyrethrin	0.16 ± 0.05	0.59 ± 0.10	12.44	1, 9	0.006	
12-3	Control	0.39 ± 0.21	1.82 ± 0.31	9.26	1, 7	0.019	
19	Pyrethrin	2.08 ± 048	2.97 ± 0.43	1.90	1, 9	0.201	
20	Control	2.22 ± 0.70	1.39 ± 0.53	0.93	1, 9	0.360	

Table 7. Survivorship of <u>Culex tarsalis</u> mosquito larvae in predator exclusion cages 24 and 168 hours post treatment in permethrin or malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September 1996. Missing values were excluded due to contamination by predatory insects or because the bucket sank.

Treatment	Field	ne ne recent care a la contra de la contra de Contra de la contra d	Surviv	vorship	(177) (177)
	. There are		24,	168 hours	
Malathion	· State wa	2014 Pal.	88%	76%	
Malathion	. 1		100%	96%	
Malathion	5	- information of States	91%	77%	
Malathion	5		100%	81%	
Permethrin	3		78%	44%	
Permethrin	3		88%	60%	
Permethrin	20		77%		
Permethrin	20	· · · · ·	86%	81%	
Control	2		83%	70%	
Control	2		100%	95%	
Control	4		78%	96%	
Control	4		92%	64%	
Control	6		100%	95%	
Control	6		96%		

Table 8. One way analysis of variance of the numbers of chironomid midges
(Chironomidae) collected each day in aquatic sweep nets pre- and post
application of malathion or permethrin treated and control fields on the Colusa
National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F
statistic, df = degrees of freedom, p = probability.

Treatment	Pre-	Post	F	df	p	
Malathion	97.0 ± 36.0	226.5 ± 50.7	2.77	1, 7	0.14	'
Control	19.0 ± 9.26	152.6 ± 52.4	3.02	1,7	0.126	
Permethrin	2.50 ± 1.44	' 101.2 ± 44.7	2.27	1, 7	0.175	
Control	35.5 ± 14.3	369 ± 101	5.07	1, 7	0.059	
Malathion	43.2 ±20.6	91.8 ± 27.8	1.29	1, 7	0.294	
Control	51.5 ± 17.1	471 ± 173	2.74	1, 7	0.142	
Permethrin	120.5 ± 45.3	450 ± 145	2.36	1, 7	0.169	
	Malathion Control Permethrin Control Malathion Control	Malathion 97.0 ± 36.0 Control 19.0 ± 9.26 Permethrin 2.50 ± 1.44 Control 35.5 ± 14.3 Malathion 43.2 ± 20.6 Control 51.5 ± 17.1	Malathion 97.0 ± 36.0 226.5 ± 50.7 Control 19.0 ± 9.26 152.6 ± 52.4 Permethrin 2.50 ± 1.44 ' 101.2 ± 44.7 Control 35.5 ± 14.3 369 ± 101 Malathion 43.2 ± 20.6 91.8 ± 27.8 Control 51.5 ± 17.1 471 ± 173	Malathion 97.0 ± 36.0 226.5 ± 50.7 2.77 Control 19.0 ± 9.26 152.6 ± 52.4 3.02 Permethrin 2.50 ± 1.44 $\cdot 101.2 \pm 44.7$ 2.27 Control 35.5 ± 14.3 369 ± 101 5.07 Malathion 43.2 ± 20.6 91.8 ± 27.8 1.29 Control 51.5 ± 17.1 471 ± 173 2.74	Malathion 97.0 ± 36.0 226.5 ± 50.7 2.77 $1,7$ Control 19.0 ± 9.26 152.6 ± 52.4 3.02 $1,7$ Permethrin 2.50 ± 1.44 101.2 ± 44.7 2.27 $1,7$ Control 35.5 ± 14.3 369 ± 101 5.07 $1,7$ Malathion 43.2 ± 20.6 91.8 ± 27.8 1.29 $1,7$ Control 51.5 ± 17.1 471 ± 173 2.74 $1,7$	Malathion 97.0 ± 36.0 226.5 ± 50.7 2.77 $1,7$ 0.14 Control 19.0 ± 9.26 152.6 ± 52.4 3.02 $1,7$ 0.126 Permethrin 2.50 ± 1.44 101.2 ± 44.7 2.27 $1,7$ 0.175 Control 35.5 ± 14.3 369 ± 101 5.07 $1,7$ 0.059 Malathion 43.2 ± 20.6 91.8 ± 27.8 1.29 $1,7$ 0.294 Control 51.5 ± 17.1 471 ± 173 2.74 $1,7$ 0.142

Table 9. One way analysis of variance of the number of aquatic beetles (Coleoptera) collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre-	Post	F	df	р
1	Malathion	13.67 ± 4.21	14.83 ± 1.80	0.09	1, 7	0.768
2	Control	6.00 ± 2.08	9.92 ± 2.69	0.88	1, 7	0.379
3	Permethrin	10.67 ± 1.92	7.17 ± 0.67	4.83	1,7	0.06
4	Control	3.67 ± 1.48	2.25 ± 0.79	0.88	1,7	0.38
5	Malathion	13.17 ± 3.61	5.25 ± 1.53	5.91	1, 7	0.04
6	Control	11.00 ± 1.61	14.58 ± 4.04	0.36	1, 7	0.57
20	Permethrin	31.83 ± 6.60	34.33 ± 7.98	0.04	1, 7	0.85

Table 10. One way analysis of variance of the number of dragonfly (Anisoptera) collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre-	Post	Fifth	df	р
1	Malathion	48.2 ± 10.1	53.2 ± 11.1	0.08	1,7	0.783
2	Control	20.3 ± 2.77	10.8 ± 3.67	3.26	1,7	0.114
3	Permethrin	15.0 ± 1.53	[•] 25.1 ± 7.43	0.85	1,7	0.387
4	Control	1.00 ± 0.764	3.67 ± 0.80	4.36	1,7	0.075
5	Malathion	1.83 ± 1.01	0.42 ± 0.33	3.00	1,7	0.127
6	Control	50.5 ± 23.2	38.7 ± 13.1	0.23	1,7	0.643
20	Permethrin	2.00 ± 1.32	2.67 ± 0.75	0.23	1, 7	0.648

Table 11. One way analysis of variance of the number of damsel fly (Zygoptera) nymphs collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre-	Post	F	df	р
1	Malathion	318 ± 104	343.1 ± 44.2	0.07	1,7	0.795
2	Control	39.67 ± 4.09	65.4 ± 18.2	0.92	1,7	0.369
3	Permethrin	1.67 ± 1.42	10.33 ± 2.97	3.80	1,7	0.092
4	Control	8.00 ± 5.48	40.8 ± 14.8	2.22	1,7	0.180
5	Malathion	4.50 ± 3.77	1.08 ± 0.33	1.84	1, 7	0.217
6	Control	1.50 ± 0.87	16.25 ± 3.07	10.6	1,7	0.014
20	Permethrin	3.83 ± 2.68	14.33 ± 3.93	3.04	1, 7	0.125
		1			1.34	

Table 12. One way analysis of variance of the number of mayfly (Ephemeroptera) nymphs collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	P . 199
1	Malathion	94.5 ± 15.3	71.0 ± 9.85	1.79	1, 7	0.222
2	Control	61.2 ± 20.3	12.58 ± 3.85	11.35	1, 7	0.012
3	Permethrin	26.67 ± 4.19	·33.0 ± 13.9	0.09	1, 7	0.767
4	Control	1.17 ± 0.93	9.75 ± 4.40	1.76	1, 7	0.226
5	Malathion	3.17 ± 0.60	0.50 ± 0.13	37.33	1, 7	0.001
6	Control	50.5 ± 14.2	46.0 ± 9.69	0.07	1, 7	0.798
20	Permethrin	121.2 ± 44.3	114.8 ± 23.0	0.02	1,7	0.891

Table 13. One way analysis of variance of the number of waterboatmen (Hemiptera: Corixidae) collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

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Field	Treatment	Pre Mean	Post Mean	F	df	р	
1	Malathion	9.33 ± 7.34	9.67 ± 4.72	0.00	1,7	0.969	
2	Control	173.0 ± 142	31.7 ± 17.9	2.12	1,7	0.189	
3	Permethrin	4.33 ± 2.49	6.83 ± 1.97	0.57	1,7	0.475	
. 4	Control	50.5 ± 23.5	89.1 ± 30.8	0.65	1,7	0.445	
5	Malathion	2.50 ± 1.04	5.42 ± 3.75	0.28	1,7	0.614	
6	Control	8.33 ± 3.63	26.50 ± 7.10	2.90	1, 7	0.132	
20	Permethrin	128.5 ± 39.9	145.0 ± 42.4	0.06	1, 7	0.813	

Table 14. One way analysis of variance of the number of backswimmers (Hemiptera: Notonectidae) collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р
1	Malathion	0.50 ± 0.00	4.08 ±1.78	1.89	1,7	0.212
2	Control	26.2 ± 24.7	20.67 ± 7.87	0.08	1, 7	0.790
3	Permethrin	7.50 ± 4.19	8.83 ± 1.80	0.12	1, 7	0.736
4	Control 3.	3.17 ± 1.59	20.58 ± 9.08	1.71	1, 7	0.233
5	Malathion	0.17 ± 0.17	1.25 ± 0.38	3.62	1, 7	0.099
6	Control	0.83 ± 0.83	2.92 ± 1.80	0.60	1, 7	0.463
20	Permethrin	13.33c2.42	17.00 ± 5.45	3.11	1, 7	0.121

Table 15. One way analysis of variance of the number of snails collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	· df	р	
1	Malathion	0.67 ± 0.67	1.25 ± 0.31	3.87	1,7	0.09	
2	Control	2 Control 1.17 ± 0.7	1.17 ± 0.73	6.33 ± 3.11	1.28	1,7	0.296
З	Permethrin	2.17 ± 1.09	2.83 ± 0.69	0.29	1, 7	0.607	
4	Control	Control 0±0	1.42 ± 0.62	2.40	1, 7	0.165	
. 5	Malathion	Malathion	0 ± 0	0.08 ± 0.08	0.47	1, 7	0.516
6	Control	0.33 ± 0.33	1.92 ± 0.78	1.86	1, 7	0.215	
20	Permethrin	3.17 ± 1.88	4.92 ± 1.32	0.58	1, 7	0.470	

Table 16. Results of one way analysis of variance of the dried biomass of aquatic invertebrates collected each day in aquatic sweep nets pre- and post application of malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA in September and October 1996. Pre-treatment and post-treatment mean abundances (± Standard Error), F = F statistic, df = degrees of freedom, p = probability.

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Field	Treatment	Pre Mean	Post Mean	F	df	р	
1	Malathion	0.42 ± 0.07	0.43 ± 0.06	0.02	1,7	0.90	in R
2	Control	0.64 ± 0.23	0.32 ± 0.08	2.60	1, 7	0.151	
3	Permethrin	0.17 ± 0.04	0.24 ± 0.04	1.69	1, 7	0.235	
4	Control	0.11 ± 0.01	0.21 ± 0.05	2.15	1, 7	0.186	
5	Malathion	0.04 ± 0.01	0.05 ± 0.01	0.36	1, 7	0.569	
6	Control	0.22 ± 0.04	0.30 ±0.03	2.00	1,7	0.201	
20	Permethrin	0.40 ± 0.16	0.53 ± 0.05	1.08	1,7	0.333	
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Table 17. The pre-treatment and post-treatment (± standard error) number of mayfly nymphs (Ephemeroptera) collected in daily dipper collections in malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Des Maser	DeatHean		-10	
Field	Treatment	Pre Mean	Post Mean	F	df	Р
1	Malathion	1.00 ± 0.58	5.50 ± 2.26	1.82	1,7	0.219
2	Control	1.00 ± 0.58	12.67 ± 7.63	1.09	1,7	0.331
3	Permethrin	1.33 ± 0.88	9.17 ± 4.99	1.14	1,7	0.320
4	Control	1.33 ± 0,88	7.83 ± 4.09	1.17	1,7	0.316
5	Malathion	0.33 ± 0.33	2.50 ± 0.72	4.07	1,7	0.084
6	Control	2.00 ± 1.00	4.17 ± 1.74	0.68	1,7	0.437
20	Permethrin	17.3 ± 11.7	70.0 ± 13.6	6.13	1,7	0.042

Table 18. The pre-treatment and post-treatment (± standard error) number of dragonfly nymphs (Anisoptera) collected in daily dipper collections in malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa CO, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р
1	Malathion	17.0 ± 11.2	30.17 ± 4.90	1.66	1, 7	0.239
2	Control	26.3 ± 16.3	19.33 ± 4.13	0.32	1,7	0.587
3	Permethrin	$2.00 \pm 0.5B$	'18.00 ± 6.82	2.57	1, 7	0.153
4	Control	31.0 ± 16.9	21.67 ± 5.47	0.47	1, 7	0.516
5	Malathion	18.33 ± 9.17	11.50 ± 3.80	0.70	1, 7	0.431
6	Control	22.00 ± 7.57	13.17 ± 5.17	0.95	1, 7	0.362
20	Permethrin	13.7 ± 12.2	27.3 ± 12.1	0.50	1,7	0.504

Table 19. The pre-treatment and post-treatment (± standard error) number of midge larvae (Chironomidae) collected in daily dipper collections in malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa CO, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	P
1	Malathion	3.33 ± 2.03	9.83 ± 5.98	0.54	1,7	0.487
2	Control	1.33 ± 0.33	12.00 ± 4.97	2.15	1,7	0.186
3	Permethrin	1.33 ± 0.67	11.83 ± 4.48	2.55	1,7	0.154
4 Control		8.33 ± 5.24	4 8.83 ± 2.20	0.01 1,	1, 7	0.918
5		Malathion 2.00 ± 1.53	2.00 ± 1.53	16.33 ± 6.10	2.54	1, 7
6	Control	5.00 ± 2.52	7.17 ± 1.60	0.57	1, 7	0.474
20	Permethrin	10.67 ± 0.88	126.7 ± 42.1	3.55	1,7	0.102

Table 20. The pre-treatment and post-treatment (± standard error) number of damselfly nymphs (Zygoptera) collected in daily dipper collections in malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa CO, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р	31
1	Malathion	1.33 ± 1.33	11.83 ± 3.33	4.49	1,7	0.072	
2	Control	18.7 ± 12.4	35.2 ± 13.7	0.58	1,7	0.47	
3	Permethrin	6.00 ± 6.00	20.33 ± 7.97	1.35	1,7	0.283	
4	Control	20.7 ± 10.1	27.0 ± 9.93	0.16	1, 7	0.703	
5	Malathion	2.67 ± 2.19	6.67 ± 2.79	0.85	1,7	0.386	
6	Control	2.33 ± 1.20	9.67 ± 4.15	1.43	1,7	0.270	
20	Permethrin	19.3 ± 11.2	82.7 ± 37.6	1.3	1, 7	0.292	

Table 21. The pre-treatment and post-treatment (± standard error) number of beetle adults and larvae (Coleoptera) collected in daily dipper collections in malathion or permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa CO, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	p	•
1	Malathion	1.33 ± 0.67	2.50 ± 0.50	1.87	1,7	0.213	
2	Control	9.33 ± 4.81	5.17 ± 2.23	0.85	1,7	0.388	
3	Permethrin	1.00 ± 0.58	1.83 ± 0.48	1.10	1, 7	0.329	
4	Control	1.00 ± 0.58	1.83 ± 0.48	1.10	1,7	0.329	
5	Malathion	13.00 ± 9.54	6.33 ± 2.11	0.92	1,7	0.370	
6	Control	7.67 ± 2.19	2.50 ± 0.43	10.94	1, 7	0.013	
20	Permethrin	30.0 ± 18.1	25.17 ± 5.72	0.11	1, 7	0.749	

Table 22. The pre-treatment, and post-treatment (± standard error) number of waterboatmen (Corixidae) collected in daily dipper collections in malathion, permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa CO, CA, in fall 1996. F = F statistic, df = degrees of freedom, p = probability.

Field	Treatment	Pre Mean	Post Mean	F	df	р	1
1	Malathion	0.00 ± 0.00	0.0 ± 0.00	NA	1, 7	NA	,
2	Control	3.00 ± 2.08	2.33 ± 0.80	0.14	1, 7	0.722	
3	Permethrin	1.67 ± 0.88	2.33 ± 1.33	0.11	1, 7	0.753	
4	Control	6.00 ± 1.53	3.00 ± 0.73	4.20	1, 7	0.080	
5	Malathion	2.33 ± 1.45	1.67 ± 0.80	0.19	1, 7	0.673	
6	Control	22.0 ± 4.93	5.33 ± 3.96	6.30	1, 7	0.040	
20	Permethrin	0.33 ± 0.33	2.33 ± 1.12	1.47	1,7	0.264	

Table 23. Abundances of dead insects collected in knockdown boxes placed in pesticide-treated and control wetlands in Colusa National Wildlife Refuge. The values for each field are the sum of insects found in three plastic boxes, each measuring 1 m² in surface area. There were no significant differences between treatments.

Field	Treatment	Chironomidae	Ephydridae	Other Diptera	Odonata
P1	Malathion	34	0	0	0
P5	Malathion	5	0	0	0
P3	Permethrin	50	0	3	1
T20	Permethrin	0	26	0	0
P2	Control	6	4	0	0
P4	Control	0	. 4	3	0
P6	Control	20	0	0	0

Table 24. Repeated-measures analysis of variance on the abundances of flying insects captured in Center for Disease Control ultra-violet light traps in Colusa National Wildlife Refuge, Colusa Co., CA. Single traps were placed in two fields treated with malathion, two fields treated with permethrin, and three control fields.
'Treatment' = pesticide treatment (malathion, permethrin, or control), 'Day' = collection date. There were three collection days before treatment and three afterward at each site. df = degrees of freedom, F = F statistic, and p = probability.

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	Between Subjects			4 A () 4	-
		df	F	р	
	Treatment	2	0.833	0.515	
	Error	3			
	Within Subjects			· · · · · · · · · · · ·	
		df	F	р	
	Day	5	4.621	0.009	
	Day X Treatment	10	0.527	0.846	
	Error	15			

Figures

- Figure 1. Layout of an experimental site on the Sutter National Wildlife Refuge, Sutter County, California, indicating the location of treatment and control fields used for assessing the effect of ultra low volume pyrethrin application on the abundance and biomass of aquatic macro-invertebrates in June, 1996.
- Figure 2. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in treatment field T2 C1 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied.
- Figure 3. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in control field T2 C3 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to other fields.
- Figure 4. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in treatment field T12 C1 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied.

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- Figure 5. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in control field T12 C3 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to other fields.
- Figure 6. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in control field T19 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied.
- Figure 7. The number of macro-invertebrates of different taxa collected each day in aquatic sweep net collections in treatment field T20 on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to other fields.
- Figure 8. Total number of aquatic macro-invertebrates collected each day in pooled sweep net collections from the first replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.
- Figure 9. Total number of aquatic macro-invertebrates collected each day in pooled sweep net collections from the second replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.

Figure 10. Total number of aquatic macro-invertebrates collected each day in pooled sweep net collections from the third replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.

- Figure 11. Dried biomass of aquatic macro-invertebrates in pooled sweep net collections from the first replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.
- Figure 12. Dried biomass of aquatic macro-invertebrates in pooled sweep net collections from the second replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.
- Figure 13. Dried biomass of aquatic macro-invertebrates in pooled sweep net collections from the third replicate of paired pyrethrin treated and control fields on the Sutter National Wildlife Refuge, Sutter Co, CA, in June 1996. The arrow indicates when pyrethrin was applied to the treatment field.

Figure 14. Layout of treatment and control fields on the Colusa National Wildlife Refuge, Colusa County, California, used for assessing the effect of ultra low volume application of malathion and permethrin on the abundance and biomass of aquatic macro-invertebrates, September and October, 1996.

- Figure 15. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the first malathion treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion was applied.
- Figure 16. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the first control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.
- Figure 17. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the first permethrin treated field on the Colusa. National Wildlife Refuge, Colusa Co, CA. The arrow indicates when permethrin was applied.
- Figure 18. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the second control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.
- Figure 19. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the second malathion treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion was applied.

Figure 20. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the third control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.

Figure 21. Time series of the number of organisms of different taxa collected each day in aquatic sweep net collections in the second permethrin treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when permethrin was applied.

Figure 22. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the first malathion treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion was applied.

Figure 23. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the first control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.

Figure 24. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the first permethrin treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when permethrin was applied.

- Figure 25. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the second control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.
- Figure 26. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the second malathion treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion was applied.
- Figure 27. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the third control field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when malathion and permethrin were applied to the treatment fields.
- Figure 28. Time series of the number of organisms of different taxa collected each day in aquatic dipper collections in the second permethrin treated field on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicates when permethrin was applied.
- Figure 29. Total number of organisms collected each in aquatic sweep net collections each day in the first pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when malathion was applied.

Figure 30. Total number of organisms collected each in aquatic sweep net collections each day in the first pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when permethrin was applied.

Figure 31. Total number of organisms collected each in aquatic sweep net collections each day in the second pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when malathion was applied.

Figure 32. Total number of organisms collected each in aquatic sweep net collections each day in the second pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when permethrin was applied.

Figure 33. Total number of organisms collected each in aquatic dipper collections each day in the first pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when malathion was applied.

Figure 34. Total number of organisms collected each in aquatic dipper collections each day in the first pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when permethrin was applied.

- Figure 35. Total number of organisms collected each in aquatic dipper collections each day in the second pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when malathion was applied.
- Figure 36. Total number of organisms collected each in aquatic dipper collections each day in the second pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, CA. The arrow indicated when permethrin was applied.
- Figure 37. Dried biomass of aquatic invertebrates collected in aquatic sweep nets each day in the first pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when malathion was applied.
- Figure 38. Dried biomass of aquatic invertebrates collected in aquatic sweep nets each day in the first pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.
- Figure 39. Dried biomass of aquatic invertebrates collected in aquatic sweep nets each day in the second pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when malathion was applied.

Figure 40. Dried biomass of aquatic invertebrates collected in aquatic sweep nets each day in the second pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.

Figure 41. The total number of flying insects collected each night in Center for Disease Control (CDC) ultra-violet light traps in the first pair of malathion treated and control fields on the Coluşa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.

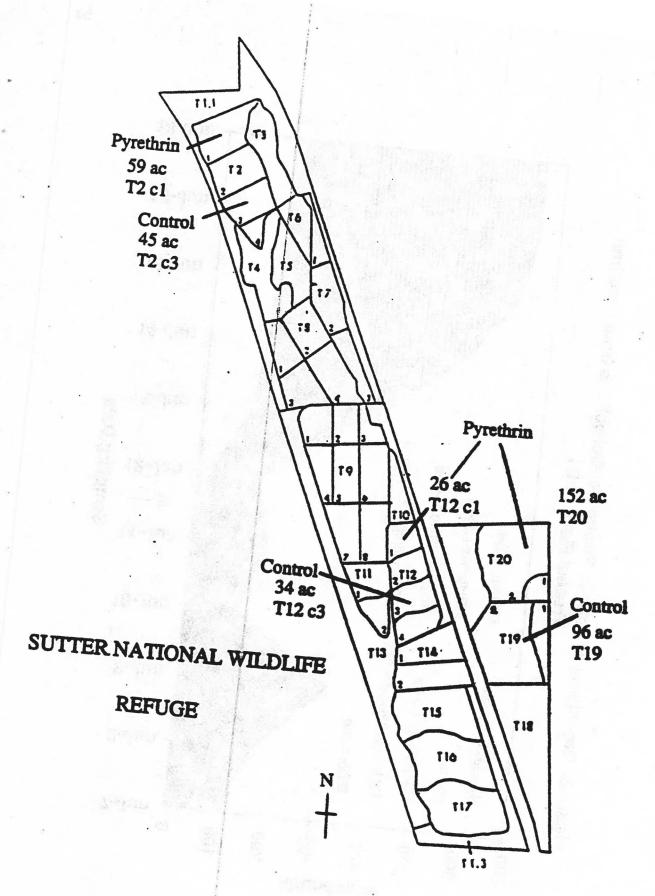
Figure 42. The total number of flying insects collected each night in Center for Disease Control (CDC) ultra-violet light traps in the first pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.

Figure 43. The total number of flying insects collected each night in Center for Disease Control (CDC) ultra-violet light traps in the second pair of malathion treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.

Figure 44. The total number of flying insects collected each night in Center for Disease Control (CDC) ultra-violet light traps in the second pair of permethrin treated and control fields on the Colusa National Wildlife Refuge, Colusa Co, California, in September and October, 1996. The arrow indicates when permethrin was applied.

Figure 45. The proportions of various taxa of flying insects collected in CDC ultra-violet light traps in fields on the Colusa National Wildlife Refuge, Colusa Co., California. Fields P1 and P5 were treated with malathion, P3 and T20 were treated with permethrin, and P2, P4, and P6 were controls. Pie charts labeled '-pre' show data averaged over three days before pesticides were applied, and 'post' indicates data averaged over three days post-application. 'Moths' = Lepidoptera, 'Mosquito' = Culicidae, 'Beetle' = Coleoptera, 'Crane Fly' = Tipulidae, 'Midges' = Chironomidae, 'Small flies' were Diptera less than 4 mm long, 'True Flies' were larger Diptera not belonging to families previously listed, and 'Misc.' included other insects such as Hemiptera.

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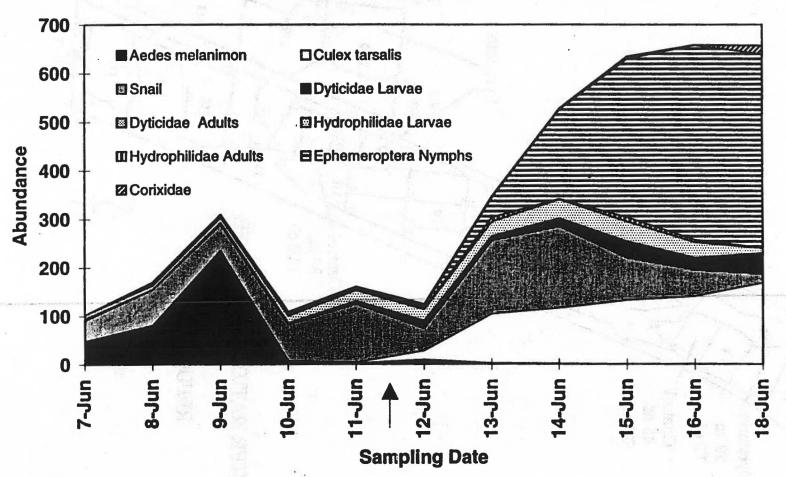


Figure 2. Organisms in Aquatic Sweep Net Collections from Pyrethrin Treated Field T2 C1

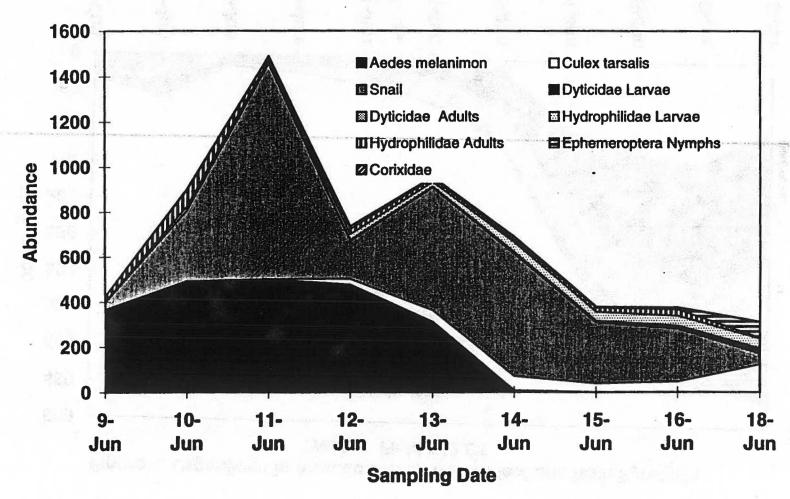


Figure 3. Organisms in Aquatic Sweep Net Collections from Control Field T2 C3

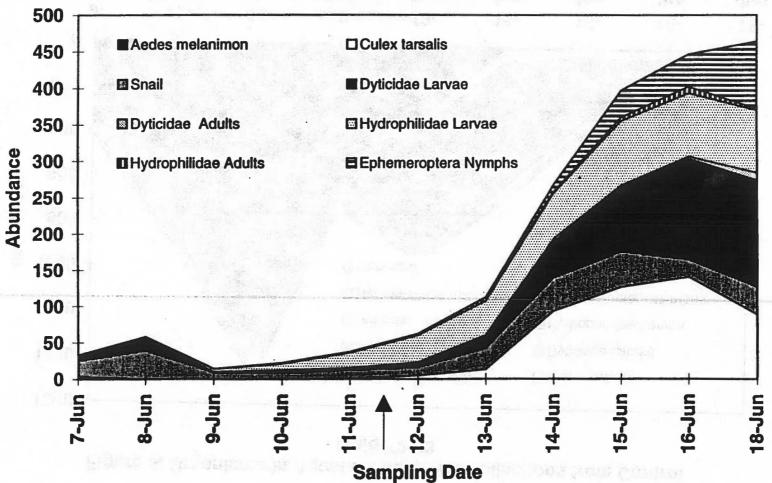


Figure 4. Organisms in Aquatic Sweep Net Collections from Pyrethrin Treated Field T12 C1

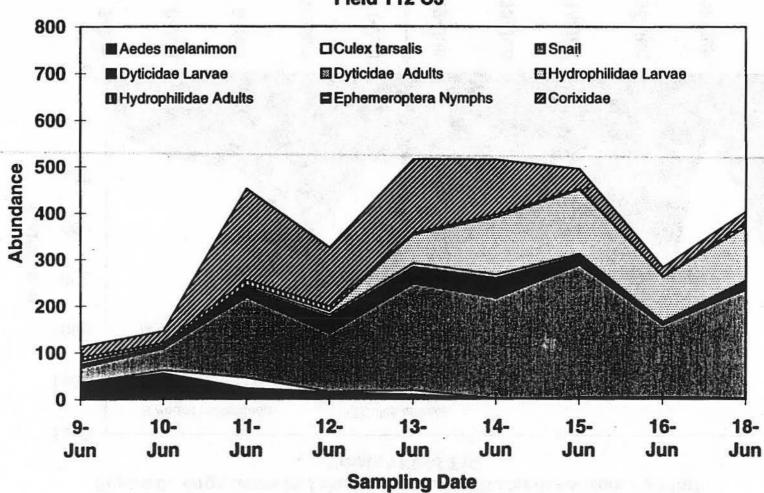


Figure 5. Organisms in Aquatic Sweep Net Collections from Control Field T12 C3

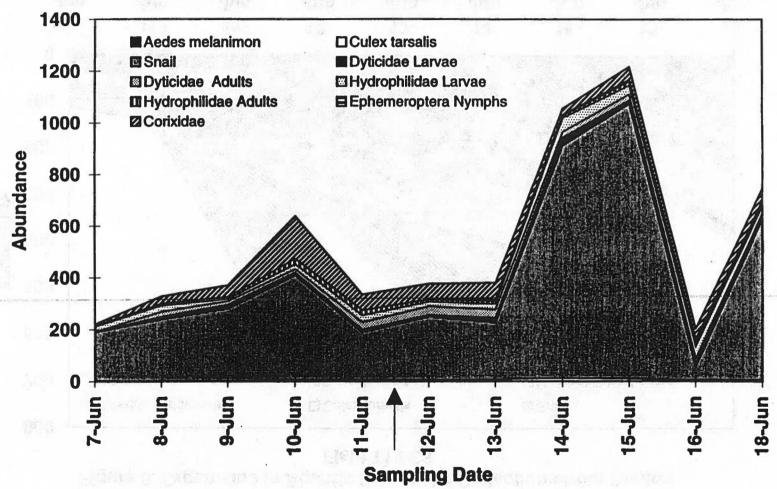


Figure 6. Organisms in Aquatic Sweep Net Collections from Pyrethrin Treated Field T19

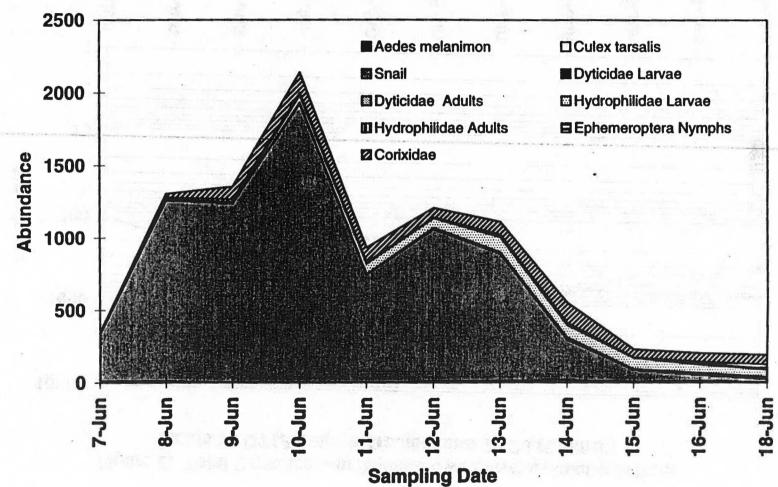


Figure 7. Organisms in Aquatic Sweep Net Collections from Control Field T20

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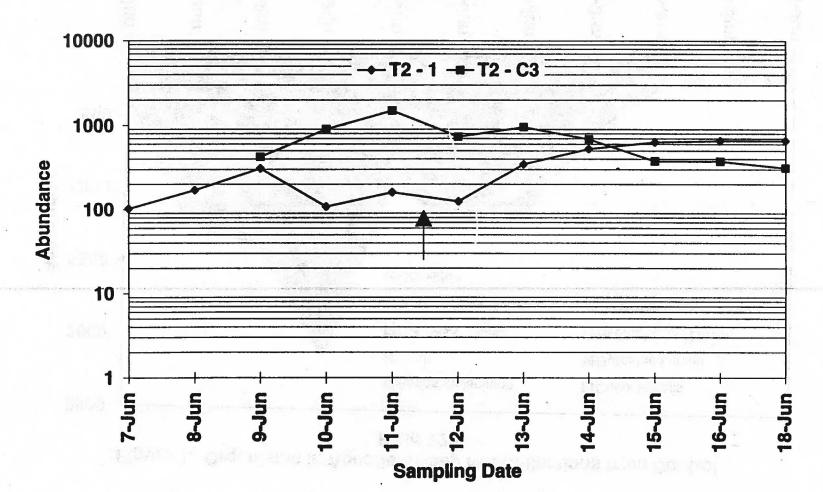
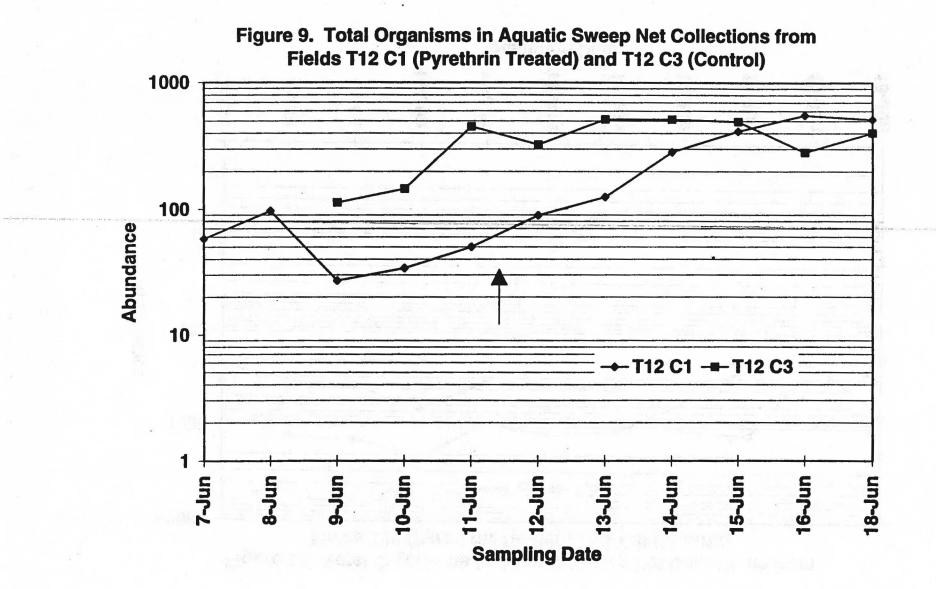
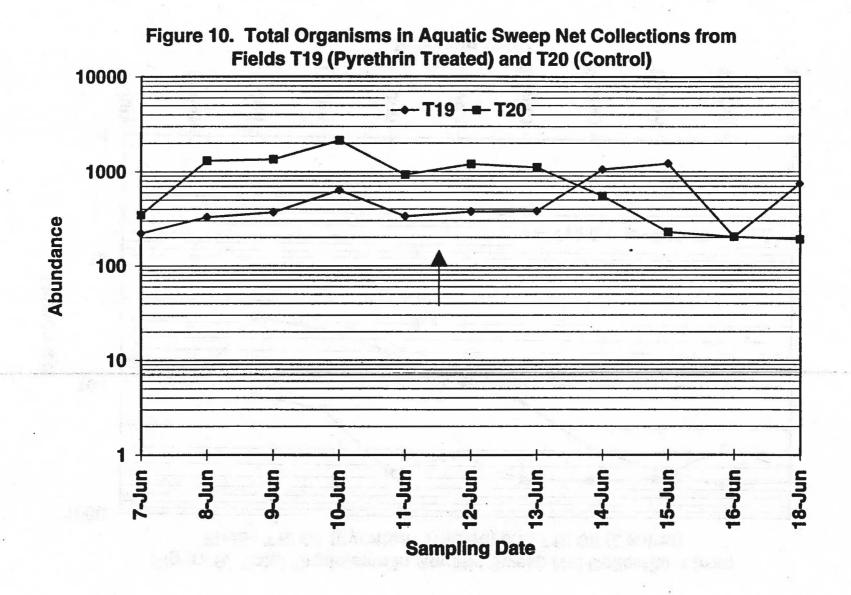
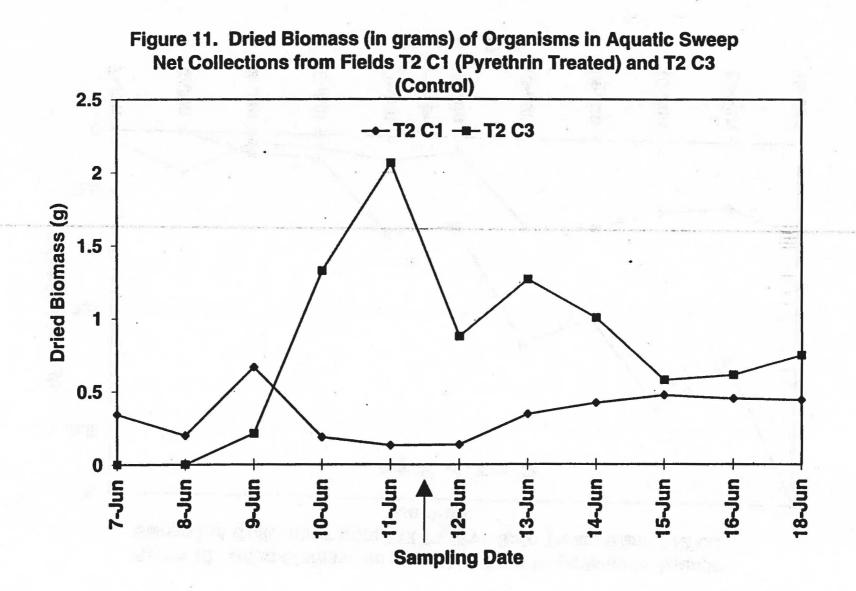
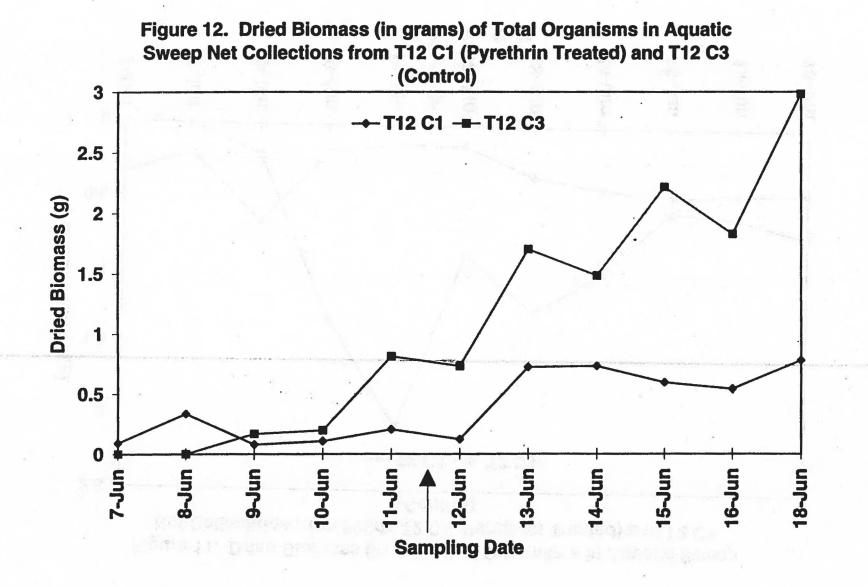


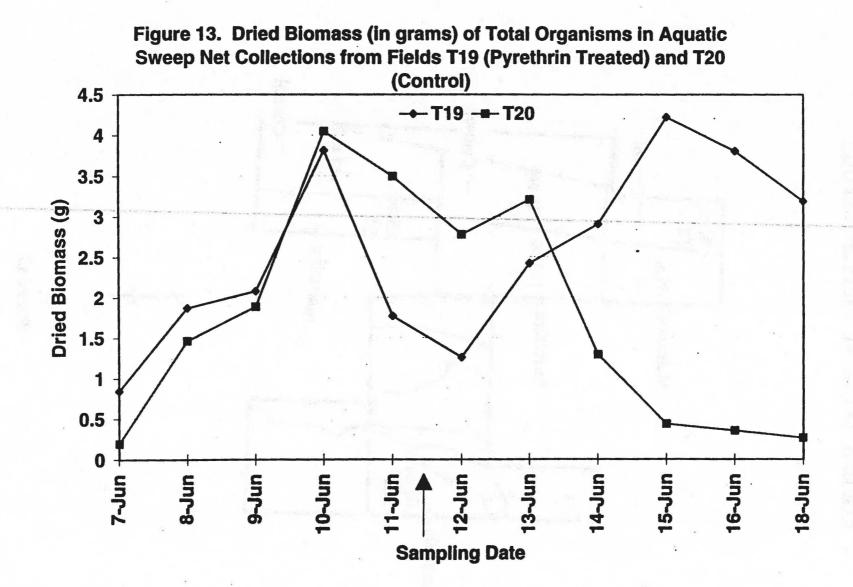
Figure 8. Total Organisms in Aquatic Sweep Net Collections from Fields T2 C1 (Pyrethrin Treated) and T2 C3 (Control)



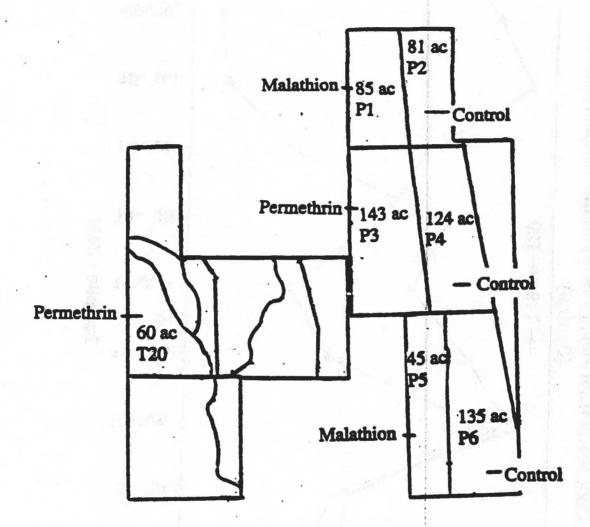








COLUSA NATIONAL WILDLIFE REFUGE



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Figure 14

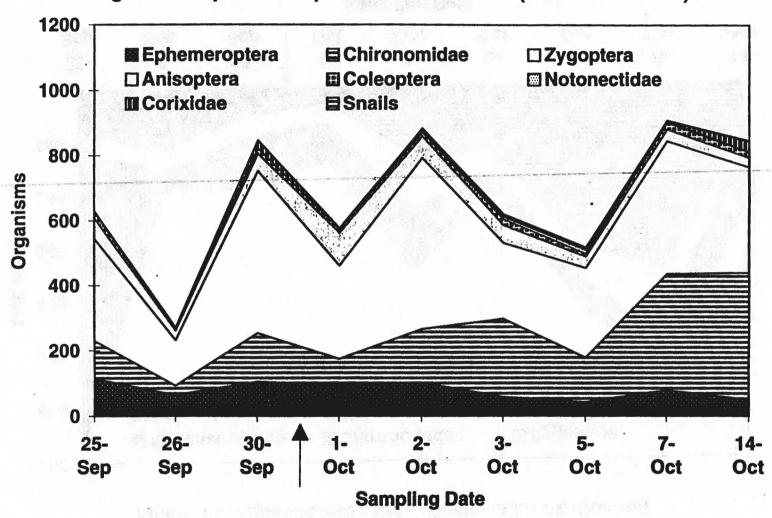


Figure 15. Aquatic Sweep Net Collection for P1 (Malathion Treated)

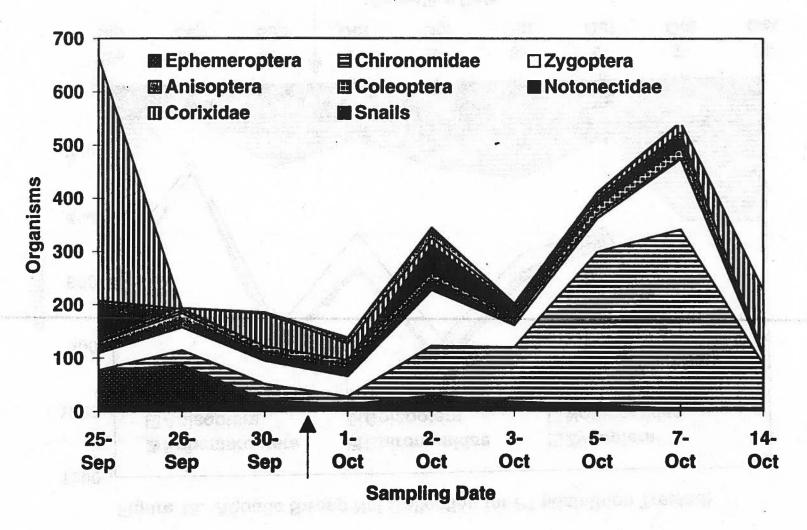
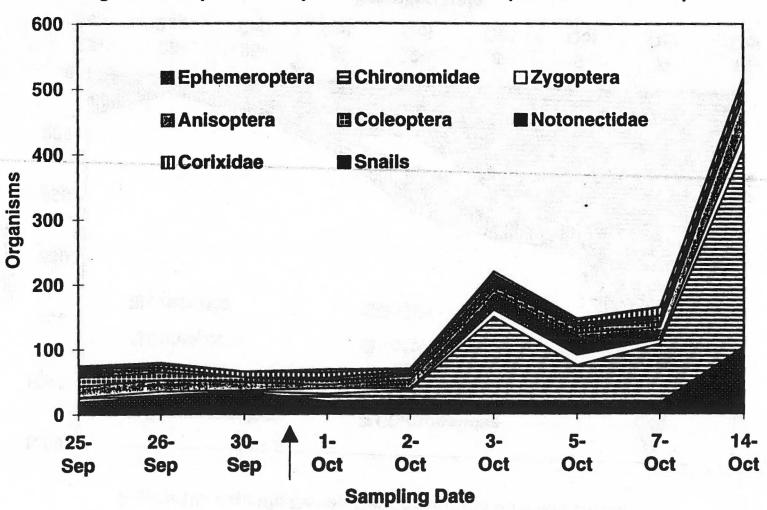


Figure 16. Aquatic Sweep Net Collections for P2 (Control)





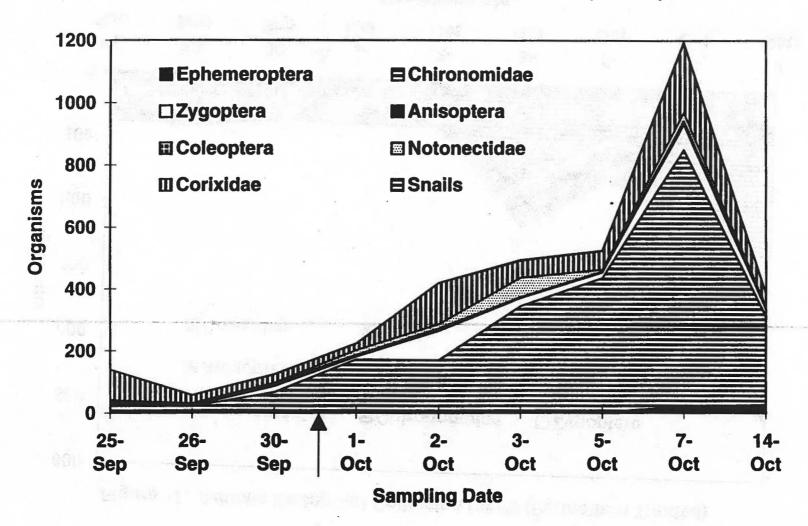


Figure 18. Aquatic Sweep Net Collections for P4 (Control)

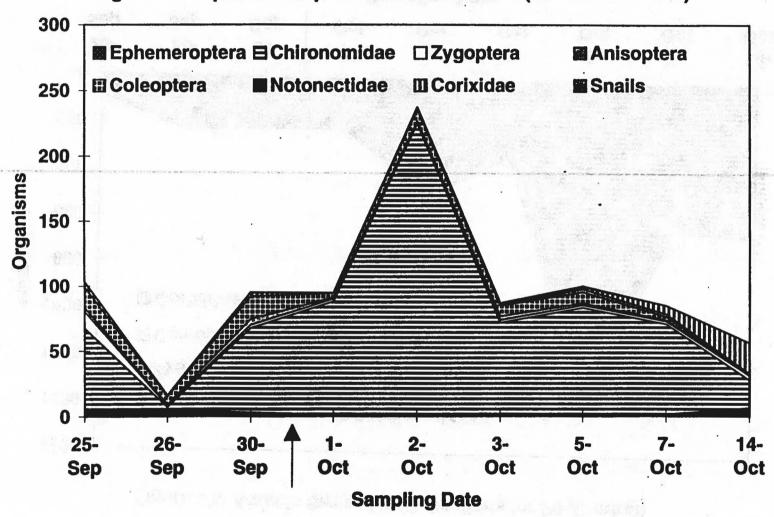


Figure 19. Aquatic Sweep net collections for P5 (Malathion treated)

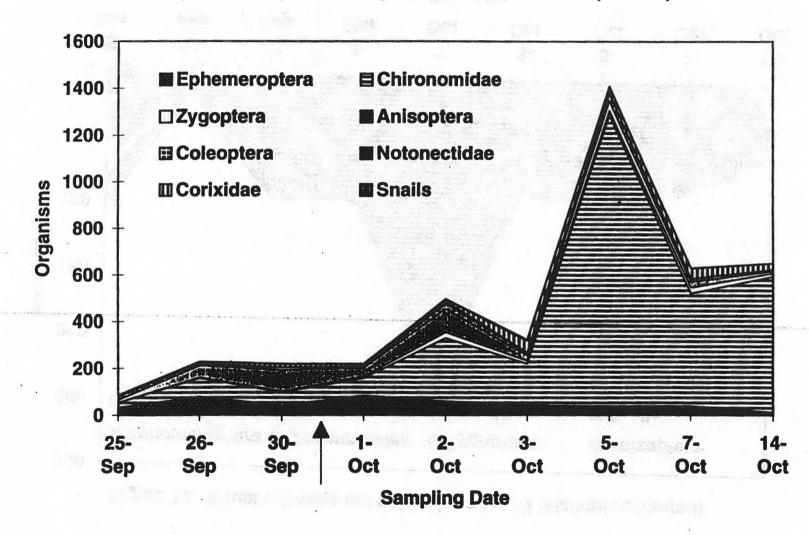


Figure 20. Aquatic Sweep Net Collections for P6 (Control)

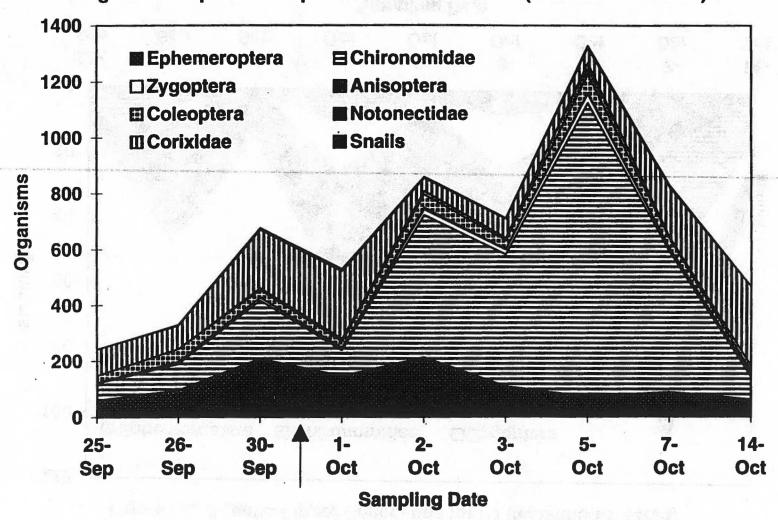


Figure 21. Aquatic Sweep Net Collections for T20 (Permethrin Treated)

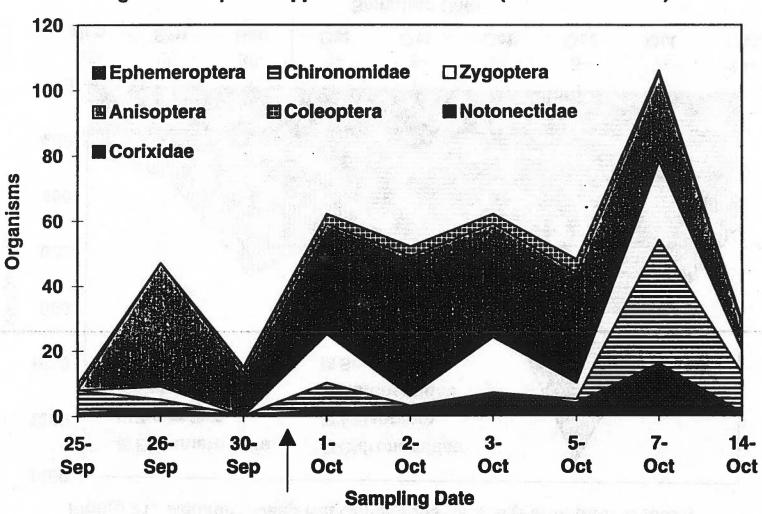


Figure 22. Aquatic Dipper Collections for P1 (Malathion Treated)

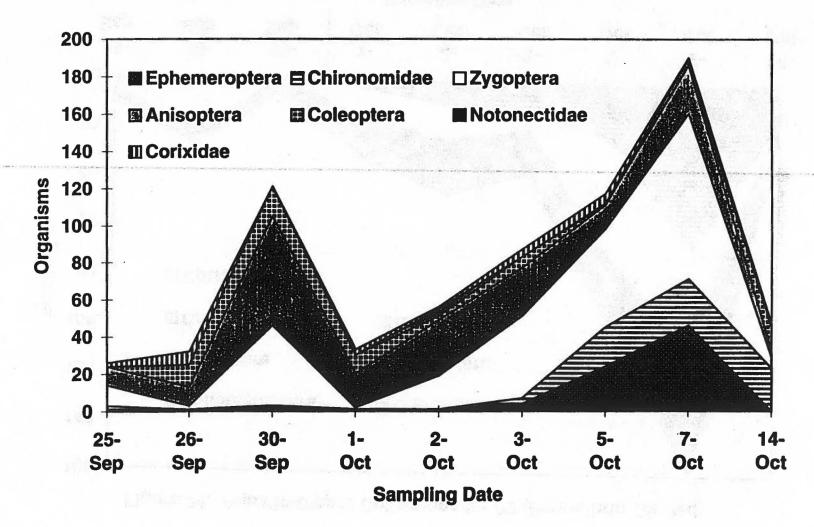


Figure 23. Aquatic Dipper Collections for P2 (Control)

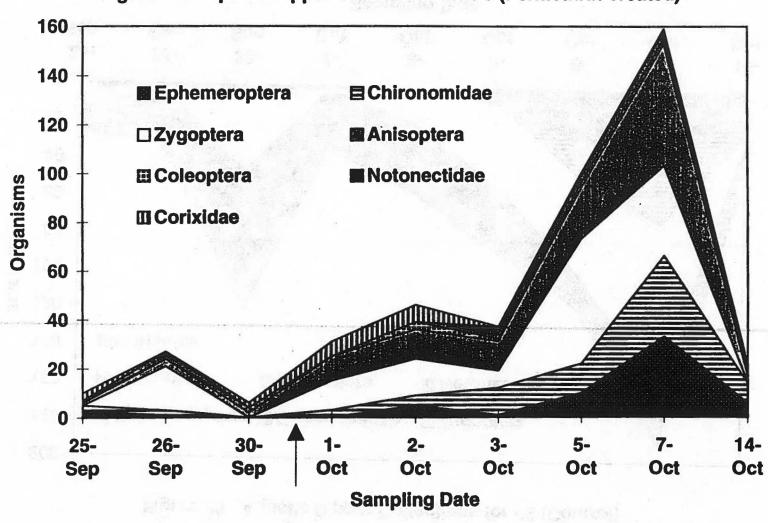


Figure 24. Aquatic Dipper Collections for P3 (Permethrin Treated)

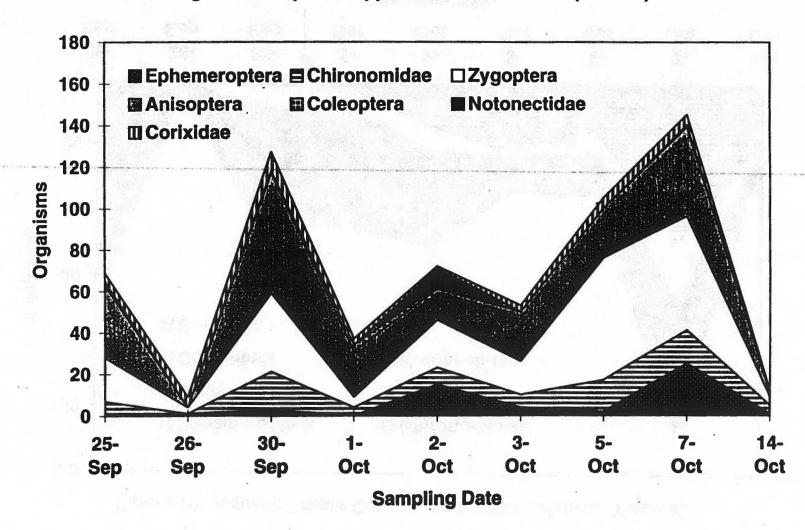


Figure 25. Aquatic Dipper Collections for P4 (Control)

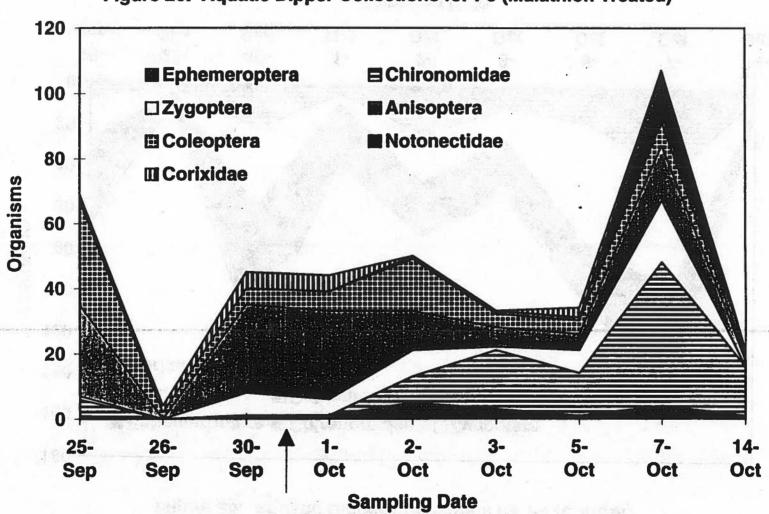


Figure 26. Aquatic Dipper Collections for P5 (Malathion Treated)

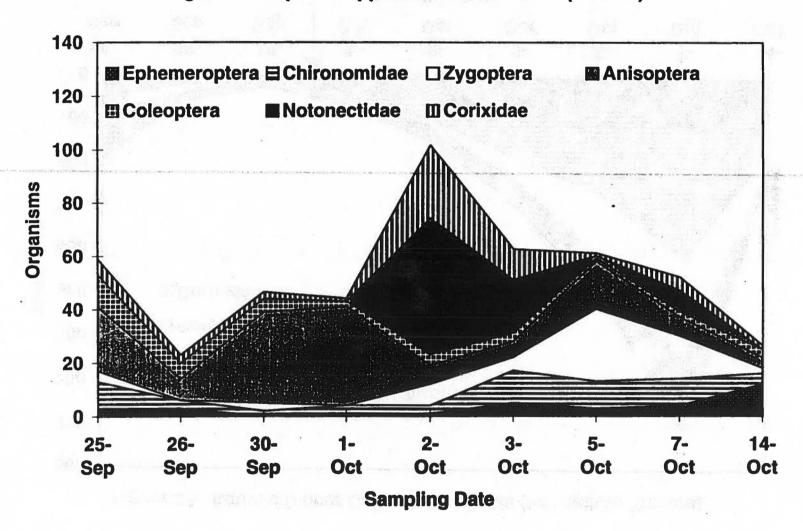


Figure 27. Aquatic Dipper Collections for P6 (Control)

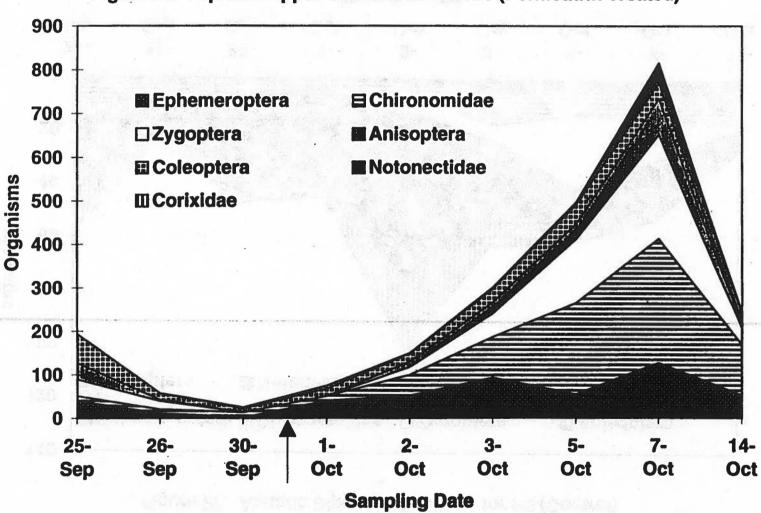


Figure 28. Aquatic Dipper Collections for T20 (Permethrin Treated)

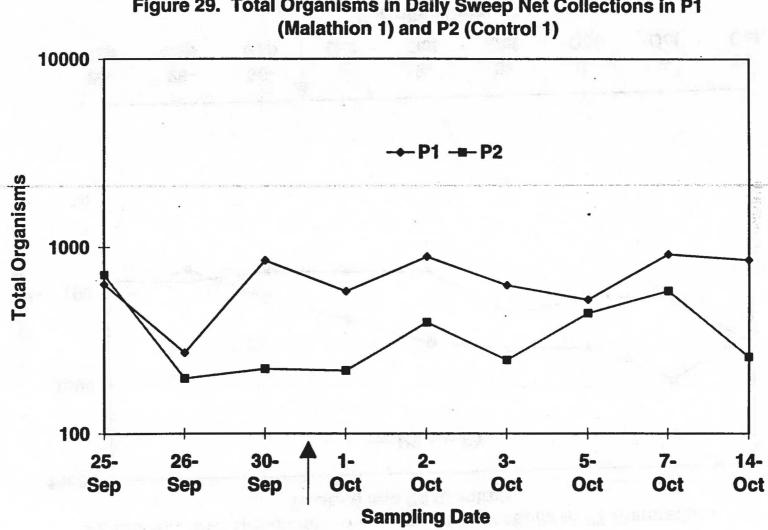
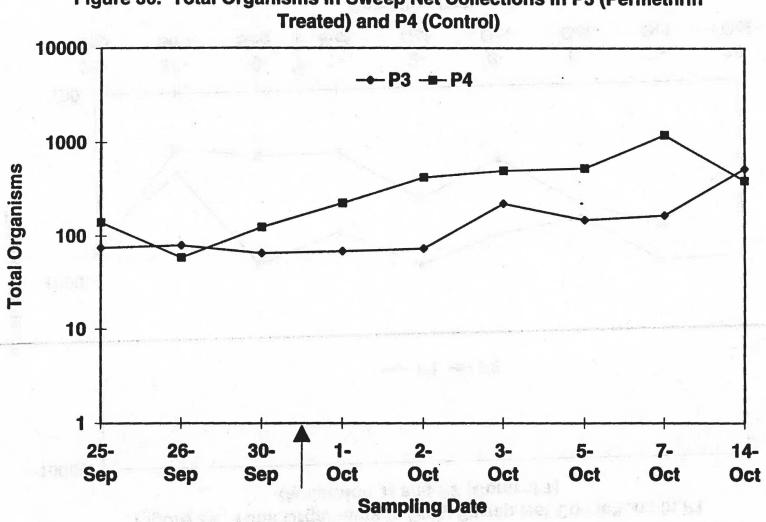


Figure 29. Total Organisms in Daily Sweep Net Collections in P1

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2.1

Figure 30. Total Organisms in Sweep Net Collections in P3 (Permethrin

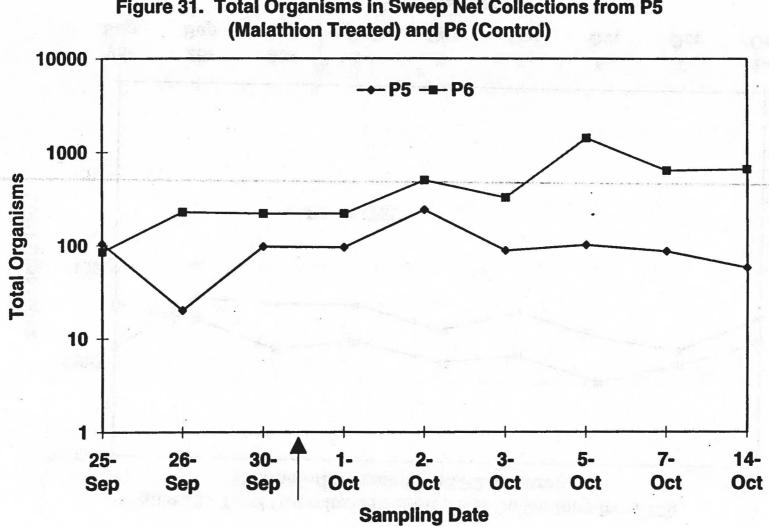
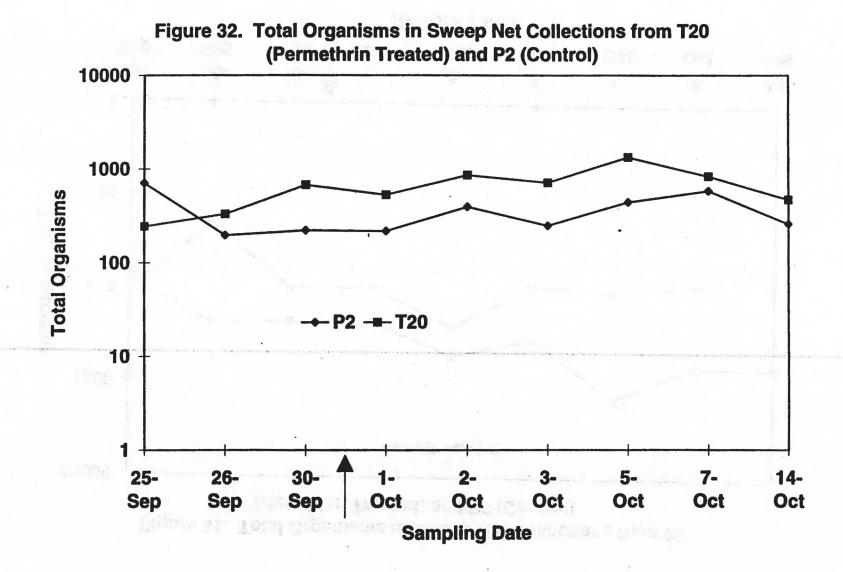
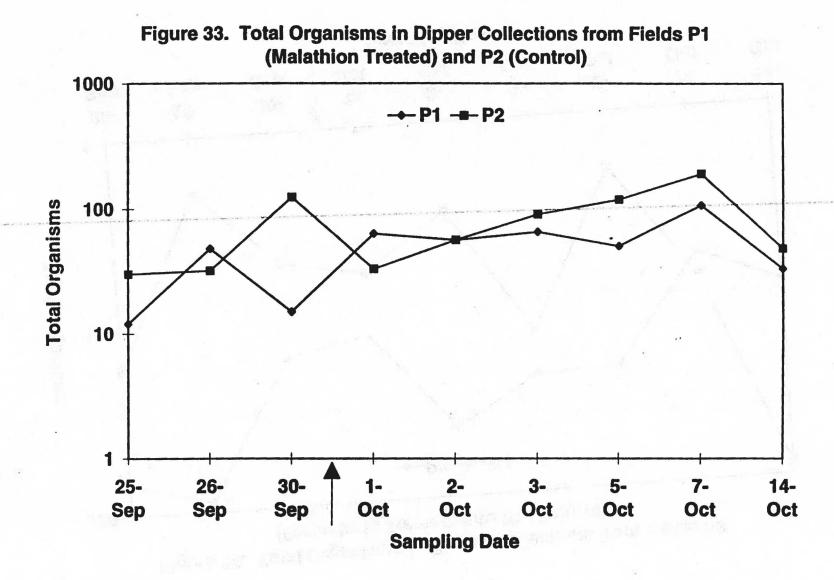
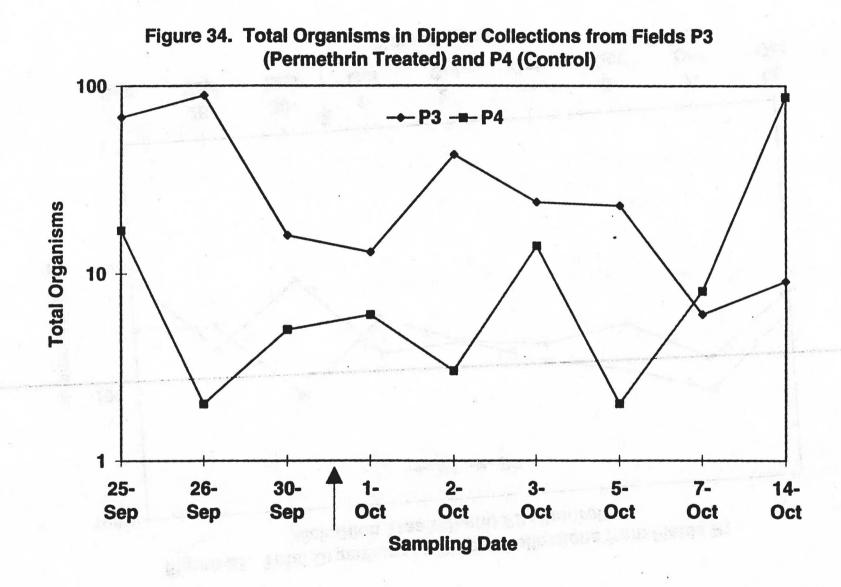


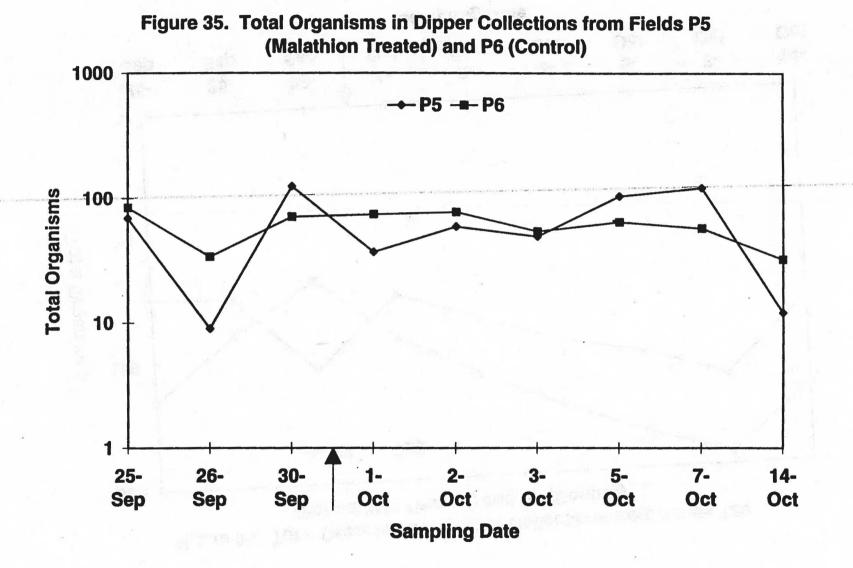
Figure 31. Total Organisms in Sweep Net Collections from P5





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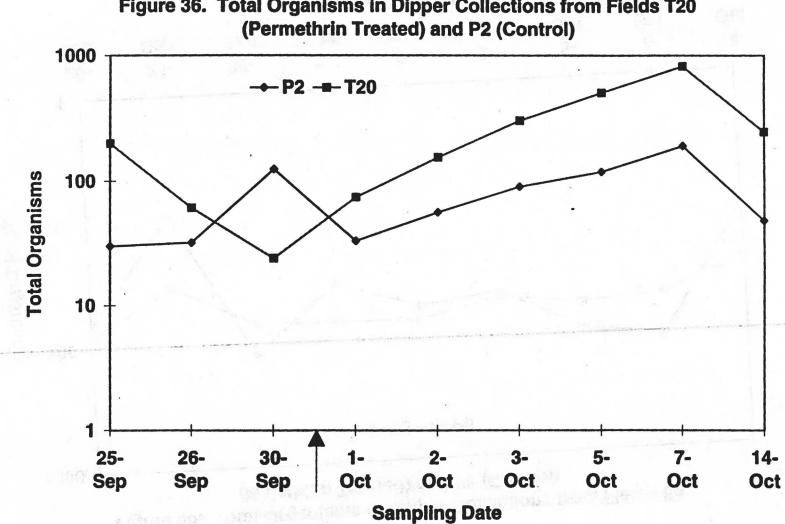


Figure 36. Total Organisms in Dipper Collections from Fields T20

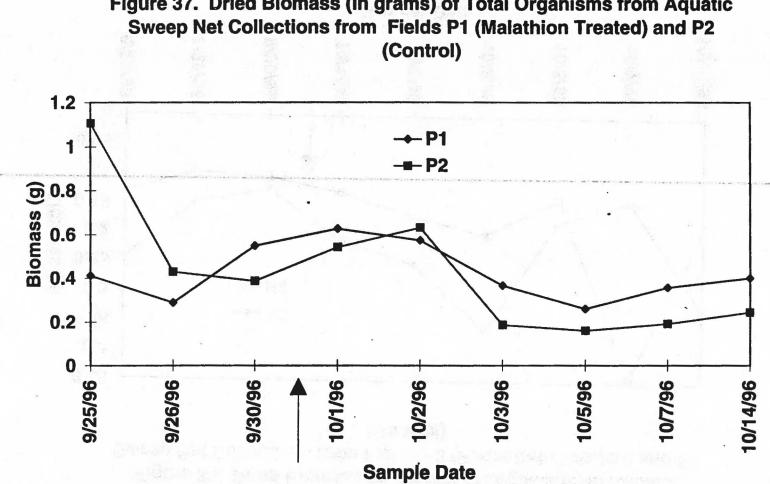
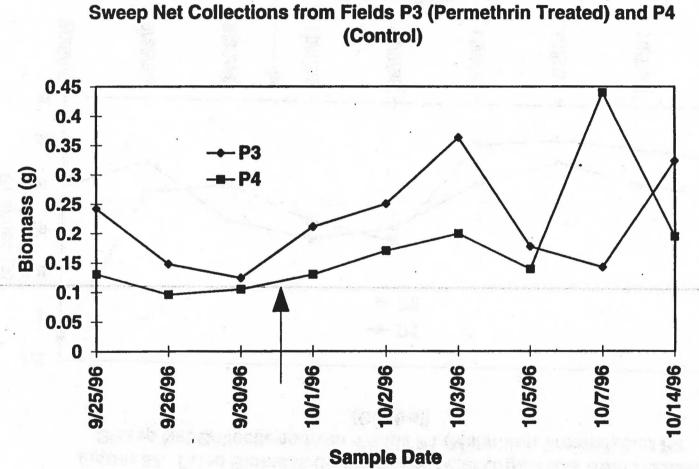


Figure 37. Dried Biomass (in grams) of Total Organisms from Aquatic

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Figure 38. Dried Biomass (in grams) of Organisms in Aquatic Sweep Net Collections from Fields P3 (Permethrin Treated) and P4

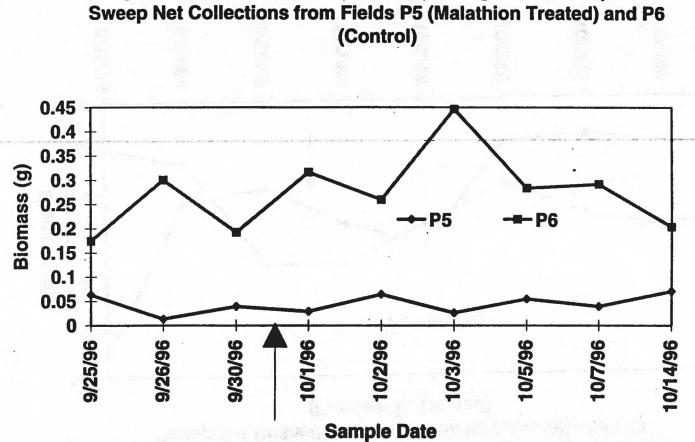
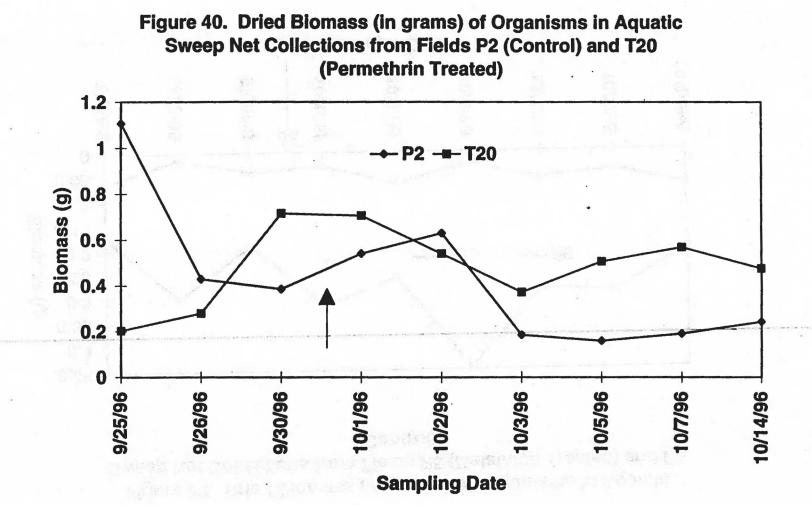
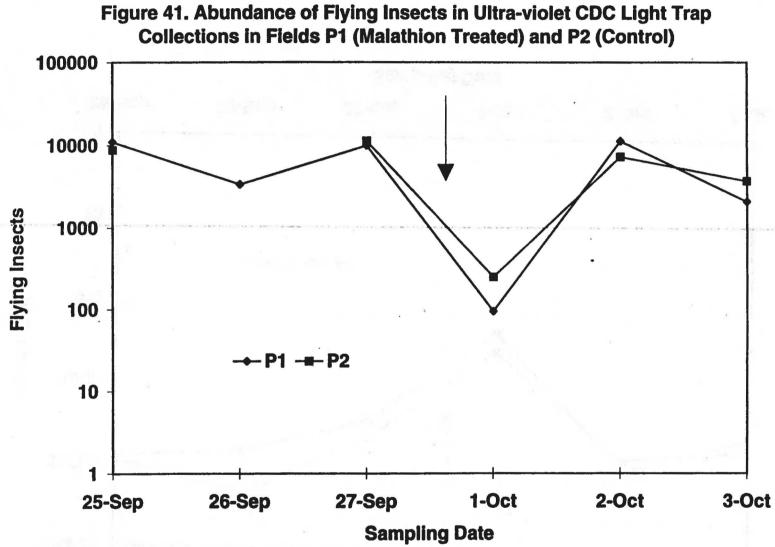


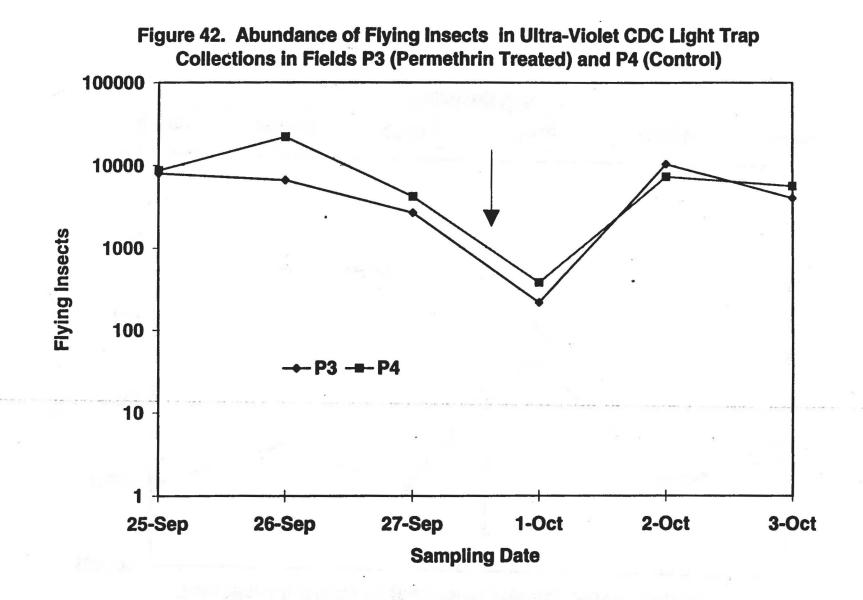
Figure 39. Dried Biomass (in grams) of Organisms in Aquatic Sweep Net Collections from Fields P5 (Malathion Treated) and P6

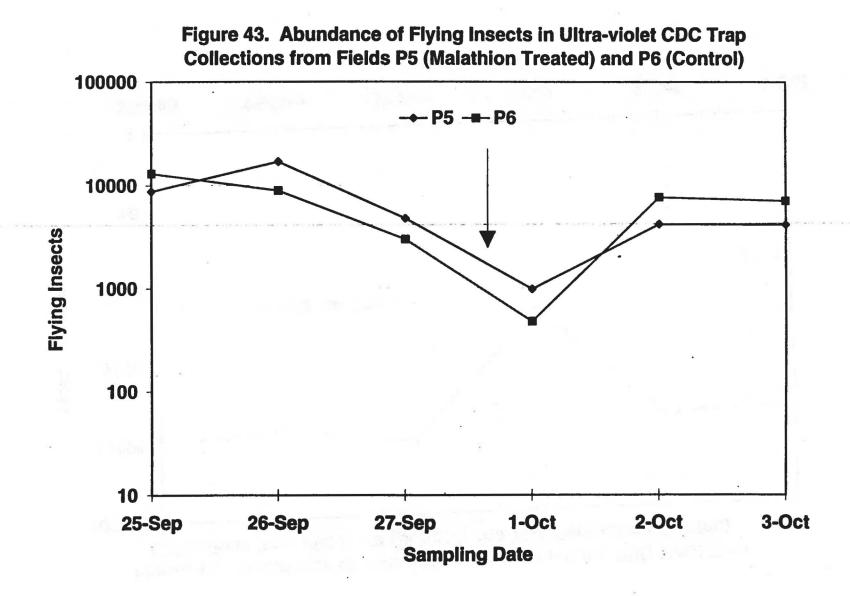
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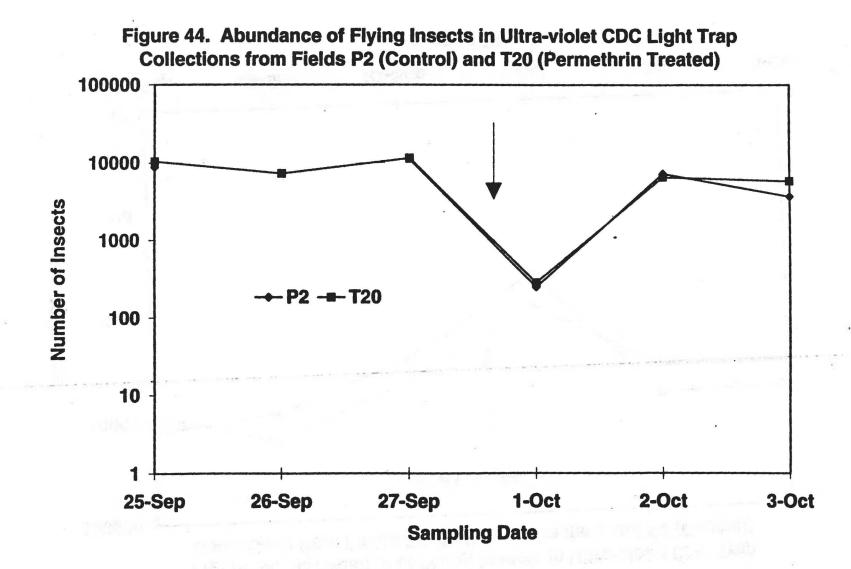
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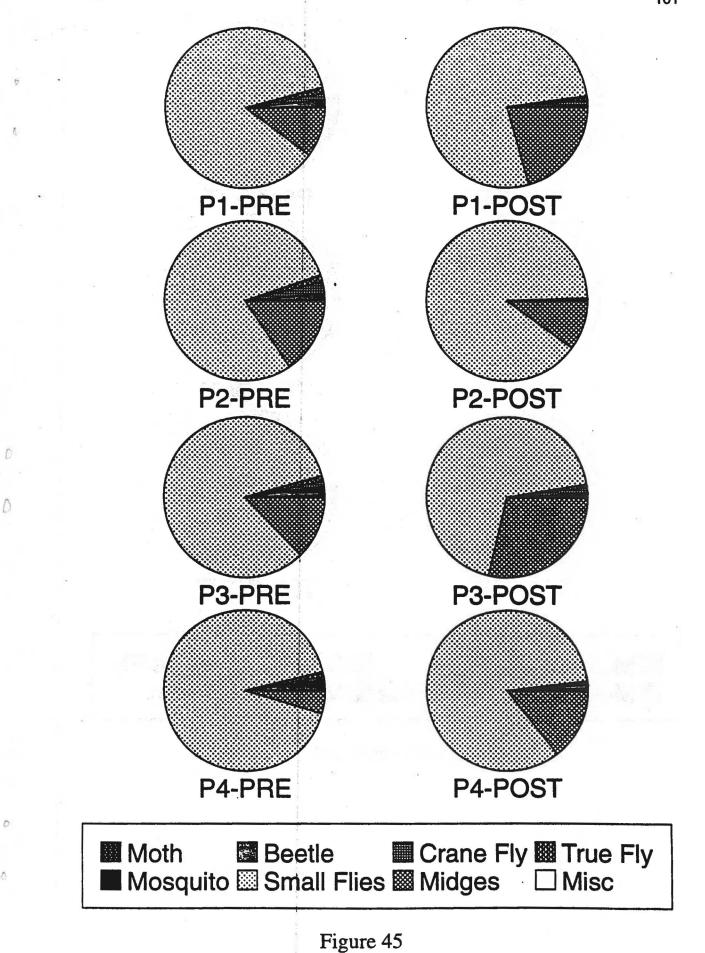


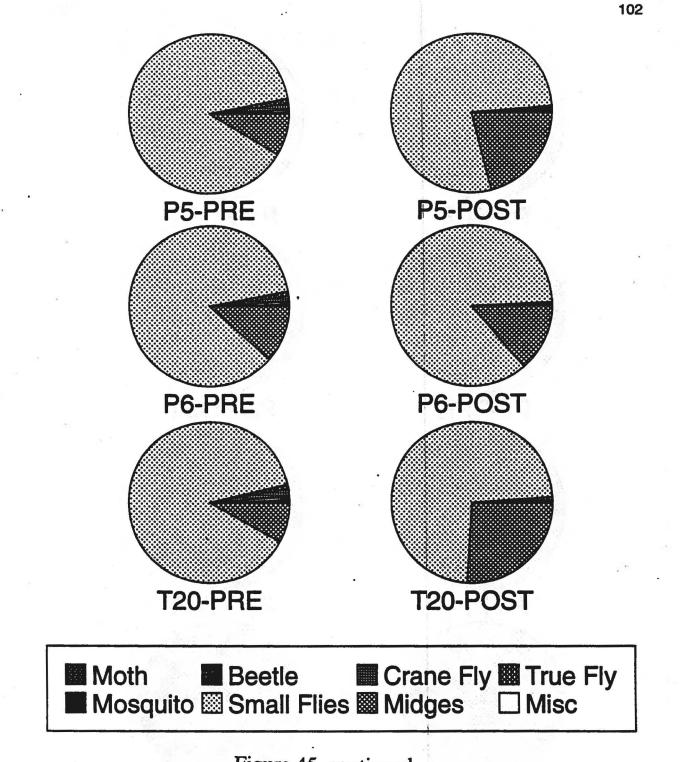












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Figure 45, continued

From FY97 Submission

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VII. BUDGET

VII.A. Previously Allocated Expe	nditures				
FY 1995 Operational		\$ 3	31,000		
FY 1996 Operational, U.C. Davis		\$1	04,059		
Funds Requested for Next FY					
Personnel Costs Salary and Benefits: U.C. Davis Staff Research benefits @ 25%		\$	35,118 8,780		
U.C. Davis Post-Graduate benefits @ 25%	Researcher		34,176		
San Francisco Bay NWR Bi	ologist		8,544 15,000		
Sacramento Field Office Bio	-		5,000		
sub-total	0		,	\$1 (06,618
Travel:					
Truck rental \$510/mo x 3 m mileage @ .24/mi x 5290 m		\$	1,530		
\$1270 x 3 mos		\$	3,810		
6 overnight stays @ \$80/nig sub-total	ght x 2 rooms	\$	960	\$	6,300
Supplies:					
(emergence traps, aquatic no boots, stakes, sample vials, sample bottles, computer su sub-total	preservatives, batter	ries, water	ts,	\$	3,000
Equipment: no cost: already availal	ble in the laboratory				
Indirect Cost (25.5% off-campus rate on U.C. D	Pavis Direct Cost of	\$ 95,918)		\$ 2	24,459
14 E-mai/	confirmed aly tical	9/30/9	6	\$1 4	40,377
NO AN	aly ti cal				

