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# EVALUATION OF SYNTHETIC HYDROCARBONS FOR MARK–RECAPTURE STUDIES ON THE RED MILKWEED BEETLE

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**Abstract**—This study evaluates the potential for using blends of synthetic hydrocarbons in mark–recapture studies of insects. To test the durability of hydrocarbons, we applied a blend of five straight-chain hydrocarbons ($C_{24}$, $C_{25}$, $C_{26}$, $C_{28}$, $C_{30}$) to detached elytra of the red milkweed beetle, *Tetraopes tetrophthalmus* (Forster) (Coleoptera: Cerambycidae), mounted the elytra on pins, and placed them in an exposed location outdoors. The amount of hydrocarbons on the elytra did not change over time, even after two months of exposure to sun and rain. Synthetic hydrocarbons applied to the elytra of living beetles did not significantly influence their longevity or mating success in a laboratory study, and the amounts of hydrocarbons did not change with age. The invariability of hydrocarbon ratios over time suggests that blends could provide a nearly infinite variety of ratios to mark individual insects uniquely and indelibly with a hydrocarbon "fingerprint." This technique offers a convenient, safe, and durable means of individually marking insects and may find application in field studies of larger bodied insects that are long-lived and sedentary.

**Key Words**—Dispersal, marking techniques, population dynamics, cuticular hydrocarbons, Cerambycidae, *Tetraopes tetrophthalmus.*

## INTRODUCTION

Mark–recapture techniques are commonly used to estimate population densities of animals and study their dispersal behavior (Hagler and Jackson, 2001). For this research, it is essential that markings be easy to apply and detect, be durable, and have no adverse effect on the development, behavior, or longevity of subjects

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(Southwood and Henderson, 2000). A variety of effective marking techniques have been developed, each with its own drawbacks. For example, materials most often used for marking insects (paints, dyes, physical tags) are easily detected, but can fade, rub off, or dislodge, and may inhibit movement of small insects or render subjects conspicuous to vertebrate predators (Southwood and Henderson, 2000; Hagler and Jackson, 2001). Mutilation marking for insects would involve clipping appendages or damaging the cuticle and would be appropriate only for heavily sclerotized insects or those with large wings (Hagler and Jackson, 2001). Application of many of these markers involves handling the insects, which subsequently may influence their dispersal behavior (Singer and Wedlake, 1981).

Fluorescent dyes can be applied as dusts to groups of insects (Crumpacker, 1974), but may have sublethal effects (Labrecque, 1975). Radioactive isotopes (e.g., $^{14}C$ and $^{65}Zn$) were once popular for marking insects (Service, 1993) but have been replaced by trace elements of low toxicity, such as rubidium, that also have the advantage of persisting in tissues (Hagler and Jackson, 2001). Rubidium levels may rapidly decline, however, once the marked insect is removed from the Rb-containing host (Fleischer et al., 1986), and high levels of exposure can affect developmental rate of larvae and kill adults (Stimmann, 1973; Culin, 1986). These rare elements are also inconvenient markers because they require extensive sample preparation, and detection by rather costly atomic absorption spectrophotometry (Hagler and Jackson, 1998). Immunoproteins can be used to mark groups of small insects, but also involve relatively complicated laboratory assays and are rapidly degraded by rain or high humidity, limiting their utility for some field studies (Hagler and Jackson, 1998).

Our study examines the potential for using blends of synthetic long-chain hydrocarbons, which are structurally stable and nonvolatile, to mark insects for field research. Long-chain hydrocarbons occur naturally in the cuticular wax layer of insects and serve as contact pheromones in some species (Howard and Blomquist, 1982). We tested the durability of hydrocarbon markers with a field study, and also tested for negative affects on the mating success and longevity of our model insect, the red milkweed beetle, *Tetraopes tetrophthalmus* (Forster) (Coleoptera: Cerambycidae). This beetle is endemic to North America and common on its milkweed hosts (Yanega, 1996).

## METHODS AND MATERIALS

We prepared a blend of synthetic hydrocarbons (Aldrich, Milwaukee, Wisconsin, USA) by mixing stock solutions of 0.05 g of tetracosane ($C_{24}$), pentacosane ($C_{25}$), hexacosane ($C_{26}$), octacosane ($C_{28}$), and triacontane ($C_{30}$) in 1 ml of hexane, and combining 20-$\mu$l aliquots of each standard in a 4:2:1:2:4 ratio, respectively. Preliminary analysis of hydrocarbon profiles of *T. tetrophthalmus* of both

sexes by gas chromatography – mass spectrometry revealed there were no major peaks that overlapped with those of the synthetic compounds.

To test the durability of hydrocarbon markers, we removed elytra from field-collected *T. tetrophthalmus* (sex not determined), mounted them individually on insect pins, and arrayed the pinned elytra on a square corkboard (30 $cm^2$). Half of the elytra were treated with 2 $\mu$l of hydrocarbon solution ($N = 25$), and the other half with 2 $\mu$l of hexane as a control ($N = 25$). The board was then placed flat on the rooftop of our laboratory building where it was exposed to full sunlight and weather. The board was left in place for 55 days between June 11 and August 5, 2000, during which time daily maximum temperatures averaged $29 \pm 3^{\circ}$C; there were 15 cloudless days, and 17.3 cm of rainfall (Illinois State Water Survey, Department of Natural Resources, University of Illinois at Urbana-Champaign).

We extracted hydrocarbons from the elytra after 0, 1, 7, 30, and 55 days ($N = 5$ elytra per date and treatment) by dipping them in 0.5 ml of hexane for 5 min (see Millar and Sims, 1998). We spiked extracts with 2 $\mu$l of a 0.01g/ml docosane solution as an internal standard (docosane does not naturally occur on the cuticle of *T. tetrophthalmus*). We quantified amounts of compounds (as fractions of the amount of internal standard) with a Hewlett-Packard 5973 mass spectrometer interfaced with a HP 6890 gas chromatograph, with an HP-5MS (cross-linked 5% phenyl-methyl siloxane) capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 $\mu$m) in splitless mode, with helium as the carrier gas. The injection port was maintained at 250°C, and the oven temperature was ramped from 200 to 240°C at 5°C/min, then 240 to 250°C at 2°C/min. We tested for changes over time in the total amount of synthetic hydrocarbons (the five compounds combined) by one-way analysis of variance (ANOVA; SAS Institute, 2001) with day being the main effect, and tested for changes in relative proportions of individual compounds by two-way ANOVA with main effects being date and compound.

To determine whether our blend of synthetic hydrocarbons influenced longevity and mating success of *T. tetrophthalmus*, we placed six females and four males (collected from the field) in each of five aluminum screen cages (30 cm on a side), and applied 2 $\mu$l of hydrocarbon blend to the elytra of half of each sex, and 2 $\mu$l of hexane to the other half. To indicate treatments, we marked beetles with a dot of enamel paint of different colors (Testors, Phoenix Model Co., Brooksville, Florida, USA). In each cage, we provided a fresh milkweed umbel and foliage in water for food; cages were kept in the laboratory. We made hourly observations between 08:00 and 17:00 hr for as long as beetles lived to measure mating success (number of times mating) and longevity. In the field, *T. tetrophthalmus* are active from early morning to late afternoon (personal observations), and males and females may remain in copula for more than an hour (McCauley, 1982). The influence of hydrocarbon marking on mating success and longevity was tested by two-way ANOVA with main effects being chemical treatment and beetle sex, and cage as a random effect. To evaluate the durability of hydrocarbons on living beetles, we

extracted the hydrocarbon-marked beetles after they died, quantified amounts of hydrocarbons (as described above for elytra), and tested for changes in total amount of hydrocarbon with beetle age (time since marking) using one-way ANOVA.

We also determined whether *T. tetrophthalmus* was affected by the solvent hexane, by using an experiment of identical design to the above, but applying 2 $\mu$l of hexane to the elytra of half of the beetles of each sex, leaving the other half as controls (four cages per treatment), and testing the treatment effects on mating success and longevity by ANOVA. We present means $\pm$ 1 SE throughout.

## RESULTS

The total amount of synthetic hydrocarbons (all five compounds combined) on the pinned beetle elytra declined by only ~25% over the two month period ($0.12 \pm 0.067$, $0.11 \pm 0.028$, $0.11 \pm 0.023$, $0.079 \pm 0.013$ and $0.089 \pm 0.00025$ $\mu$g for 0, 1, 7, 30, and 55 days, respectively), but these differences were not statistically significant (ANOVA $F_{4,19} = 0.33,\ P > 0.86$). In addition, the relative proportions of the individual compounds were consistent over time, as indicated by the insignificant interaction term in the ANOVA ($F_{16,24} = 0.24,\ P = 0.99$; time effect $F_{4,24} = 1.19,\ P = 0.32$; compound effect $F_{4,24} = 9.44,\ P < 0.001$; Figure 1).

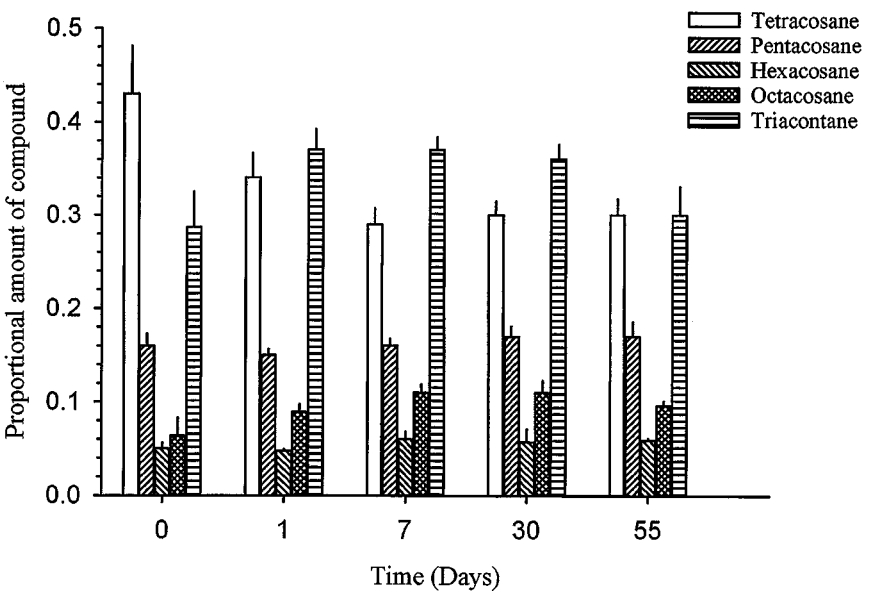


FIG. 1. Proportion of individual hydrocarbons to the total extracted from elytra of *T. tetrophthalmus* that had been exposed outdoors.

Male *T. tetrophthalmus* were observed to mate 0–18 times (average $5.9 \pm 1.33$), and females mated 0–13 times (average $4.44 \pm 0.63$) while caged in the laboratory, and the number of matings was not influenced by hydrocarbon treatment (ANOVA treatment effect $F_{1,40} = 0.23,\ P = 0.64$). Moreover, hydrocarbon treatment did not influence longevity: hydrocarbon- and hexane-treated beetles lived $12.7 \pm 1.32$ and $14.1 \pm 1.40$ days, respectively (ANOVA $F_{1,33} = 0.65,\ P = 0.43$). The total amount of synthetic hydrocarbons on marked beetles did not vary with their age, averaging $0.0365 \pm 0.017$ $\mu$g across treatments (day effect not significant; ANOVA $F_{6,13} = 0.94,\ P = 0.52$). As with the hydrocarbon-marked elytra, the relative proportion of compounds (individual peaks divided by the total) applied to living beetles did not vary among time periods of 0–4 days, 5–8 days, and 9 and 15 days (ANOVA interaction term $F_{8,14} = 0.68,\ P = 0.71$; time effect $F_{2,14} = 1.34,\ P = 0.27$; compound effect $F_{4,14} = 13.32,\ P < 0.001$).

Hexane alone did not affect mating success of *T. tetrophthalmus*, with hexane-treated and untreated beetles mating an average of $3.76 \pm 0.86$ and $3.12 \pm 0.68$ times, respectively (means not significantly different, treatment effect $F_{1,43} = 0.39,\ P = 0.53$). Solvent did not influence longevity: hexane-treated and untreated beetles lived $9.1 \pm 1.34$ and $9.9 \pm 1.42$ days, respectively (means not significantly different, treatment ANOVA $F_{1,32} = 0.15,\ P = 0.70$).

## DISCUSSION

The amounts of synthetic hydrocarbons that we had applied to beetle elytra changed little over time, even after nearly two months of exposure to high temperatures, sunlight, and rain. This invariability of hydrocarbon ratios over time suggests that blends would provide a nearly infinite variety of ratios that could be used to uniquely and indelibly mark individual insects with a synthetic hydrocarbon "fingerprint." Hydrocarbon blends are easily applied in the field with a small paintbrush or can be applied to some insects by spraying; this labeling is unlikely to influence rates of predation. It takes only a few minutes to extract hydrocarbons from each insect, and analysis requires ~15 min per sample by gas chromatography. Hydrocarbon markers could also be quickly and easily detected, without harming marked insects, by solid-phase microextraction (Shirey, 1999). In a preliminary study, we detected synthetic hydrocarbon blends from the marked elytra of dead *T. tetrophthalmus* using a 7-$\mu$m polydimethylsiloxane fiber designed to extract high molecular weight compounds (Supelco, Bellefonte, Pennsylvania, USA). Finally, the synthetic hydrocarbons we used for marking are relatively inexpensive: a kit of straight-chain alkanes ($C_5$–$C_{30}$) costs ~US$200 (Aldrich, Milwaukee, Wisconsin, USA) and would yield more than 10 liters of solution per compound, enough for a career of mark–recapture experiments.

Our study confirmed that synthetic hydrocarbons applied directly to the elytra of *T. tetrophthalmus* did not adversely affect mating success or longevity;

however, sublethal effects of hydrocarbons or solvent may influence, for example, dispersal or reproduction. In particular, this technique is probably not suitable for marking soft-bodied insects because hexane may be more readily absorbed through their cuticles. Because cuticular hydrocarbons may play a role in chemical communication among insects (Howard and Blomquist, 1982), care also must be taken in selecting synthetic hydrocarbons that will not influence mating behavior and other intraspecific interactions. Marking insects with hydrocarbons that do not naturally occur on the cuticle, however, is unlikely to influence mating behavior.

We conclude that application of blends of synthetic hydrocarbons offers an effective, convenient, and inexpensive means of uniquely marking insects for long-term field studies of population size and dispersal, and is probably best suited for marking long-lived, large bodied, and heavily sclerotized insects, such as orthopteroids, coleopterans, hymenopterans, and lepidopterans. Because detection of the marker requires chemical analysis, the technique is probably best suited for relatively sedentary insects that would be recaptured at a greater rate and so would require smaller sample sizes. The technique could be extended by using as markers branched-chained hydrocarbons to provide a greater selection of synthetic markers or halogenated hydrocarbons that can be detected with greater sensitivity by electron-capture gas chromatography.

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