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Effects of Enamel Paint on the Behavior and Survival of the Periodical Cicada, Magicicada Septendecim (Homoptera) and the Lesser Migratory Grasshopper, Melanoplus Sanguinipes (Orthoptera).

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EFFECTS OF ENAMEL PAINT ON THE BEHAVIOR AND SURVIVAL OF THE PERIODICAL CICADA, *MAGICICADA SEPTENDECIM* (HOMOPTERA) AND THE LESSER MIGRATORY GRASSHOPPER, *MELANOPLUS SANGUINIPES* (ORTHOPTERA).

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ABSTRACT

We present information compiled from several studies on the effects of methods for marking individual arthropods on their longevity and behavior. Results from our own research on effects of enamel paint marking on two insect species, the periodical cicada, *Magicicada septendecim*, and the lesser migratory grasshopper, *Melanoplus sanguinipes*, are also presented. Neither species showed any adverse survivorship or behavioral effects from marking.

The ease of use of different marking methods and their appropriateness for particular study designs are well reviewed (Southwood 1978, Walker and Winewriter 1981, Kearns and Inouye 1993). Above and beyond logistical concerns, the possible effects on the study subjects further constrains the choice of marking method. Table 1 summarizes literature reporting effects of individual marking (not mass-marking) methods on study organisms. We located sources using published reviews and electronic databases (Zoological Record, Wilson Indexes to Journal Articles, and Biological Abstracts) and included only papers containing data specifically addressing possible mortality or morbidity due to marking. Of several hundred papers examined, we found only 19 fitting these criteria. This table is neither exhaustive nor prescriptive, but rather supplements earlier reviews. Few general trends are apparent, except that marks applied topically to sclerotized parts seemed to have few deleterious effects, while dusts, powders, or, in the case of soft-bodied insects, some solvents, must be used cautiously. The lack of general trends means that controlled testing of a marking method is the only way to ensure its appropriateness for any given study.

Individual marking has proven effective in our research on periodical cicadas [*Magicicada septendecim* (L.)] and lesser migratory grasshoppers [*Melanoplus sanguinipes* (F.)], allowing us to track the behaviors of individual insects or classes of insects as they emerge, mature, mate, and die. One marking technique we have used is to apply colored enamel paint dots to the dorsal thoracic surface (Testors® lead-free gloss enamel; Testors Corp., Rockford IL, 61104), or to use paint pens (Uni®-Paint Fine Line PX-21 paint markers; Eberhard Faber Inc., Lewisburg TN 37091). Because published re-

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Table 1. Reported effects of methods for marking individual insects, grouped by general method and organized chronologically within each grouping.

Reference	Marker	Taxon	Effect*
This study	Enamel paint (xylol, propanol based)	*Magicicada septendecim* (Homoptera) *Melanoplus sanguinipes* (Orthoptera)	None
Hunter 1960	Enamel paint (xylol, propanol based)	*Laelaspis georgiae* (mite)	None
Caprio *et al.* 1990	Enamel paint (xylol, propanol based)	*Leptinotarsa decemlineata* (Coleoptera)	None
Hager and Kurczewski 1986	Enamel paint (xylol, propanol based)	*Ammophila harti* (Hymenoptera)	None§
Greenslade 1964	Enamel paint (petroleum-solvent based)	*Nebria brevicollis* (Coleoptera)	None†
Paulson and Akre 1991	Enamel paint (petroleum-solvent based)	*Formica neoclara* (Hymenoptera)	None
Dobson *et al.* 1958	Oil-based paint	*Lepthohylemia coarctata* (Diptera)	None
Dobson *et al.* 1961	Lacquer-based paint (nitrocellulose)	*Lepthohylemia coarctata* (Diptera)	Toxic
Davey 1956	Oil-based paint (Rubine Toner or Monolite Yellow in wood rosin and kerosene)	*Schistocerca gregaria* (Orthoptera)	None
Gallepp and Hasler 1975	Typewriter correction fluid	*Brachycentrus* spp. (Trichoptera)	Behavioral changes
Fales *et al.* 1964	Fingernail polish (acetone based)	*Musca autumnalis* (Diptera)	Toxic
Garcia and Paleari 1990	Fingernail polish (acetone based)	*Charidotis punctatostriata* (Coleoptera)	None
Dreyer and Baumgartner 1997	Fingernail polish (acetone based)	*Clavigralla tomentosicollis* (Hemiptera)	None
Brussard 1971	"Sharpie" marker (xylol solvent)	*Erebia epipsodea* (Lepidoptera)	None
Rieske and Raffa 1990	Acrylic paint	*Hylobius pales* (Coleoptera) *Pachylobius picivorus* (Coleoptera)	None
Caprio *et al.* 1990	Paper labels affixed with cynoacrylate glue	*Leptinotarsa decemlineata* (Coleoptera)	None
Caprio *et al.* 1990	Acetone wash, followed by paper labels affixed with cynoacrylate glue	*Leptinotarsa decemlineata* (Coleoptera)	None
Hart and Resh 1980	Plastic tags affixed with wire or elastic band	*Dicosmoecus gilvipes* (Trichoptera)	None

Sempala 1981	Fluorescent powder combined with Duco paint	*Aedes africanus* (Diptera)	Toxic
Gangwere *et al.* 1964	Colored thread or paper attached with glue	*Acheta domesticus* (Orthoptera)	None‡
Stuart 1986	Polyester fiber tied around body	*Leptothorax* spp., *Harpagoxenus* spp. (Hymenoptera)	None
Gangwere *et al.* 1964	Melted crayon, colored ink, Aniline dyes, or Titanium oxide spray	*Acheta domesticus* (Orthoptera)	None‡
Gangwere *et al.* 1964	Metallic dust	*Acheta domesticus* (Orthoptera)	None‡
Gangwere *et al.* 1964	Fluorescent powder, pigment in oil spray, Oil and Cellulose paints, dry pigment	*Acheta domesticus* (Orthoptera)	Toxic in large doses
Gangwere *et al.* 1964	Wing amputation or clipping	*Periplaneta americana* (Blattaria)	None for moderate treatment
Mead-Briggs 1964	Tarsal clipping	*Spilopsyllus cuniculi* (Siphonaptera)	None
Mascanzoni and Wallin 1986	Radar tags	*Pterostichus melanarius, P. niger, Harpalus rufipes, Carabus granulatus* (Coleoptera)	None

* "None:" no effects reported; "Toxic:" increased mortality reported; "Injury:" unspecified damage or mortality noted.
§ Pers. Comm.
† Dispersal attributable to handling effects noted.
‡ Method proved to be impermanent and of limited use.

ports contain no clear information on the toxicity of the solvents in these paints (xylene, xylol and n- propoxy propanol) we conducted experiments to determine whether this marking method has adverse effects on our study animals.

MATERIALS AND METHODS

Periodical cicadas. We compared the survivorship and mating activity of marked and unmarked adult *Magicicada septendecim* in a recently logged clearing along Route 639 in the Horsepen Lake State Wildlife Management Area, Buckingham County, VA, in mid-May 1996. Two cages were constructed by enclosing living vegetation with a 1 × 2 m piece of black fiberglass window screen; each cage was stocked with 10 unmarked and 10 marked mature, unmated female cicadas. We marked each cicada by using a flat toothpick to apply one yellow and two blue dots of Testors® enamel paint. We used no anesthetic during marking, and we were careful to prevent paint from contacting the wing articulations or the articulation between the head and thorax. The total surface area covered by paint was between 6 and 10 mm^2. Unmarked cicadas were handled in similar fashion. To allow matings, on May 20, 1996, we captured 20 sexually mature adult males from the surrounding chorus and placed 10 in each female cage.

Cages were censused on May 23, 24, 29, 30, 31, June 1, 2, and 4. At each census, we counted and removed dead cicadas within each cage, recorded whether or not they were marked or showed evidence of a mating sign (or "seminal plug;" White 1973), and preserved them in 70% ethanol. We removed, examined, and preserved all remaining cicadas at the final census.

Lesser migratory grasshoppers. We collected male and female nymphs of *Melanoplus sanguinipes* (F.), the lesser migratory grasshopper, from the grounds of the Matthaei Botanical Gardens at the University of Michigan, Ann Arbor, Michigan. We raised the grasshoppers to maturity in 50 × 40 × 40 cm fiberglass screen cages, on a diet of romaine lettuce, wheat bran and wheat seedlings *ad libitum*. Cages were kept at room temperature (20°C ± 2°), but provided with a 90 watt incandescent light bulb on a 13:11h light: dark cycle to allow for thermoregulation. Twenty one-week-old adults of each sex were chosen for marking and handled individually; half (10 of each sex) were marked with two dots of paint on the pronotum. We used four different colors of Uni®-PaintFine Line paint markers.

All 40 individuals were maintained for 6 days in a 50 × 40 × 40 cm cage under the same conditions described above. We examined the cage twice every day, once between 09:00 and 12:00 and once between 13:00 and 19:00, and recorded the paint/no-paint status of all copulating individuals, as well as any deaths.

RESULTS

Periodical cicadas. We compared the survivorship of the marked and unmarked cicadas using Cox regression analysis, implemented with the PHREG function in SAS (SAS Institute Inc., 1996). This technique allowed us to include individuals surviving at the end of our observations. The small difference in survivorship curves for marked and unmarked individuals was not statistically significant (Wald Chi-Square = –2.32, 1 d.f., p = 0.13), and the trend was in the direction of marked cicadas seeming to survive longer than unmarked cicadas. Mortality rates in the two cages differed (Wald Chi-

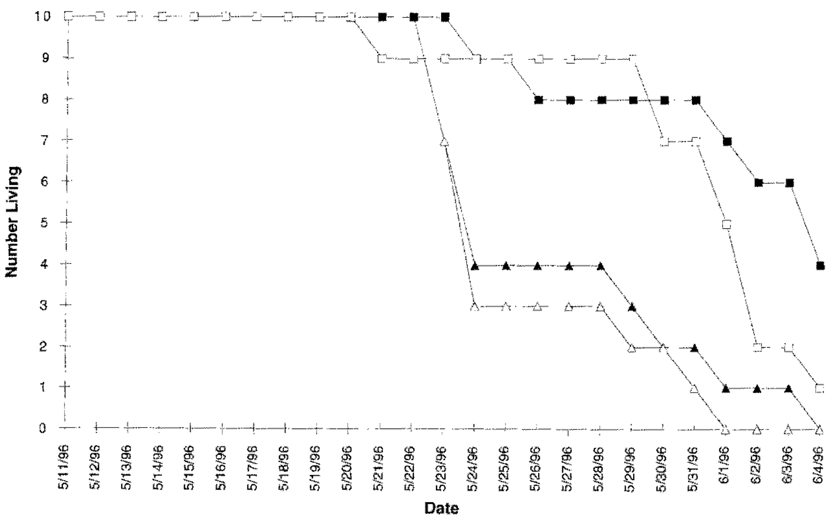

Figure 1. Survivorship of marked and unmarked cicadas in two replicate cages. Triangular and square markers indicate different replicates, filled markers indicate marked treatment, open markers indicate unmarked treatment.

Square = 14.02, 1 d.f., p = 0.0002), probably due to the death of substantial amounts of vegetation in one cage, although the increased mortality in this cage was distributed equally among marked and unmarked cicadas.

A chi-square goodness-of-fit analysis of seminal plug presence/absence data reveals no significant pattern. Marked and unmarked females were equally likely to exhibit this indicator of mating status. Data are summarized in Figure 1 and Table 2.

Lesser migratory grasshoppers. We observed 31 copulations over 6 days. Of these, 8 were between unmarked females and unmarked males, 6 were between marked females and unmarked males, 10 were between unmarked females and marked males, and 7 were between marked females and marked males. There was no difference in likelihood of marked or unmarked males or females being *in copula* (Chi-Square Goodness-of-Fit Test, χ^2=1.31, p>>0.10). During the 6 days after marking there was no mortality or apparent illness in any of the marked or unmarked individuals.

DISCUSSION

We have uncovered no evidence that careful marking with enamel paints affects cicadas' or grasshoppers' mortality during the short (< 12 day) study period. Furthermore, marking does not affect the behavior or attractiveness of female cicadas in any way that alters likelihood of mating. For the grasshoppers, paint marking appeared to have no impact on the sexual behavior of either sex.

Table 2. Results of "sperm plug" analysis, demonstrating that marking had no effect on tendency of female Magicicada septendecim to mate.

	Total Females	"sperm plug" present	χ^2	p
Females in replicate A with "sperm plug"				
Marked	10	6		
Unmarked	10	7		
Total	20	13	0.038	p>>0.1 (n.s.)
Females in replicate B with "sperm plug"				
Marked	10	5		
Unmarked	10	6		
Total	20	11	0.045	p>>0.1 (n.s.)
Females in combined replicates with "sperm plug"				
Marked	20	11		
Unmarked	20	13		
Total	40	24	0.083	p>>0.1 (n.s.)

From our experiences in the field, we can draw attention to several pitfalls best avoided when marking insects with enamel paints. First, paint applied to body parts that undergo significant flexion (such as the wings or dorsum of the thorax in flying insects) tends to wear or flake off, usually beginning a week to ten days after marking, but sometimes noticeably after only a day or two. Secondly, the pigments in enamel paints fade under daily exposure to sunlight; thus some colors may become indistinguishable within a week of marking. The difficulty in distinguishing similar pigments such as blue and violet can be exacerbated by the varying light conditions in the field; the contrast between two given pigments differs depending on atmospheric conditions, orientation of the marked insect, or time of day. Redundancy in the marking scheme alleviates this problem to some extent. Finally, enamel paint spots fade and flake off when marked specimens are preserved in alcohol (we have experience with ethanol and denatured alcohol; other commonly available alcohols probably give similar results).

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