INSECT MARKING TECHNIQUES: DURABILITY OF MATERIALS[1,2]

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ABSTRACT: Durability of 26 marking materials was tested on three species of insects for usefulness in studies requiring long-term recognition of individual insects. The three — chosen for their differences in size, cuticular surface, and habitat — were a field cricket, *Gryllus rubens*; a mole cricket, *Scapteriscus acletus* (pubescent, burrowing); and a flour beetle, *Tribolium castaneum* (small, oily). Non-water-soluble paints proved most suitable. One of these, Tech-Pen Ink, remained on all three species of test insects throughout their adult lives. The nature of the surface to which the marks were applied and marking conditions influenced how long a material adhered.

Recognition of individuals is important in many studies of insects, such as those dealing with territoriality or reproductive success. This generally requires a marking material that lasts the lifetime of the adult insect. Many art, hobby, and industrial products might serve this purpose. In most studies, whatever material is tried and works at all is used from then on. Generally no time can be spared for finding an optimal marking material. Although Walker and Wineriter (1981) tested the durability of three highly-rated materials, this study is the first extensive survey of the performance of candidate marking materials in long-term studies. We report here the durability of 26 marking materials on the pronata of three species of insects.

MATERIALS AND METHODS

Test insects were southeastern field crickets, *Gryllus rubens,* southern mole crickets, *Scapteriscus acletus,* and red flour beetles, *Tribolium castaneum.* These species were chosen because of their differences in size, cuticular surface, and habitat, and their ease of rearing. Southeastern field crickets are insects of moderate size, have smooth, shiny pronota, and live in leaf litter; southern mole crickets are larger-than-average, have pubescent pronota, and live in subterranean tunnels; red flour beetles are small, have shiny, oily pronota, and inhabit grain products.

Marking materials were selected by perusing art, hobby shop, and office supply stores, talking with students and colleagues, and corresponding with companies that might make suitable materials. The 26 materials chosen are listed in Table 1. If the product was known or suspected to be particularly

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durable, more than one color of the product, if available, was tested (viz. Tech-Pen Ink and Liquid Paper Correction Fluid).

Insects were marked as follows. A dot (mark) of each material was applied to each quadrant of the pronotum of five young adult individuals of each species (N = 20 dots/material/species). A marking system using dots was selected because the marks are easy to apply and read (Walker and Wineriter 1981). More elaborate marks, e.g. numbers or letters, can be used only on insects with large writing surfaces and have the disadvantage of making the insect highly conspicuous. Materials were applied in several ways. The bent tip of a minutin pin was used to apply dots to red flour beetles. The broken sharp tip of a wooden applicator stick was used to mark field crickets and the blunt end of a wooden applicator stick was used to mark mole crickets, except that the applicator brush was used for Liquid Paper Correction Fluid, and the marker tip was used for Pentel White Marker.

Each set of marked individuals (5 insects with 20 marks of one material) was placed in a container with the appropriate food. Mole crickets were allowed to burrow in damp sand, and flour beetles in flour. At one-week intervals food was replenished, and insects were checked for partially or wholly missing marks and or mortality. Checks continued until more than 50% of the marks were lost on living insects in a container (i.e. until the median mark was lost), or until all insects were dead.

The data were analyzed using two ranking systems. The first system determined which materials were likely to stay on throughout the adult life expectancies of the insects; the second, if marks were lost, whether marks disappeared gradually or all at once. These systems are explained fully in footnote "a" of Table 1.

RESULTS

Results are detailed in Table 1. Materials that were water-soluble when applied, though water-resistant or waterproof when dry, generally adhered poorly. Of eight such materials tested, six did not last well on any of the three insects used. A seventh material, Stroblite Daybrite Tempera, stayed on mole crickets and field crickets but not on flour beetles. The remaining material, Dupont Latex Enamel, was not durable on field crickets or flour beetles, but our data suggests it would adhere to mole crickets.

Non-water-soluble materials were more durable. Only one material, Tech-Pen Ink, was successful in marking all three insect species. For flour beetles it was not consistent, but no other material was successful in marking flour beetles, according to the criteria of this study. Two materials, Liquid Paper Correction Fluid and Nissen Metal Marker, dried before they could be applied to the flour beetles and could not be evaluated. (The amount

of material needed for a mark was small, and these paints were fast-drying.) For Creme L'Oreal Nail Accents, the results on flour beetles were inconclusive because the experiment was inadvertently terminated early; however, almost 50% of the marks were lost by the sixth week, indicating this material would not be highly rated. For mole crickets, in contrast to flour beetles, 16 of 18 non-water-soluble materials adhered well. Phosphorescent Ink PB412 did not adhere well and Pentel White Marker stayed on only two out of three times. Where more than one color of a material was used, as in Tech-Pen Ink and Liquid Paper Correction Fluid, or repetitions of the same color, as in Tech-Pen Ink orange, results were consistent for mole crickets (except for Pentel Pen as mentioned above).

Only 9 of 18 non-water-soluble materials adhered well to field crickets, 5 materials did not, and the data for 3, though inconclusive because the crickets died too soon, suggest these materials would have worked well. When more than one color of a material was used Tech-Pen Ink was consistent in results while Liquid Paper Correction Fluid was not.

Of those materials that were durable throughout the life expectancy of the three insects, evidence of peeling, chipping or flaking was rare (see Table 1). Most partial loss of marks occurred in materials used on mole crickets, fewer on field crickets, and almost none on flour beetles. Liquid Paper Correction Fluid was the only material that gradually disappeared on both mole crickets and field crickets.

Non-water-soluble materials had varying qualities that affected their ease of application and "scorability" over time. Most of these materials, except some colors of Tech-Pen Ink, had to be mixed well before application because the pigment tended to separate from the base. If this was not done, the marks made with these paints were less pigmented and more difficult to score. Many materials, such as Gams Printer's Ink, were very fluid and bled when applied, sometimes forming irregular and poorly pigmented marks that were difficult to score. A few materials, such as Tech-Pen Ink and Pactra Hi-Glo, were more viscous and formed regular, easy-to-score marks. Two materials, Liquid Paper Correction Fluid and Pentel Pen, faded over time. Buff, a color of Liquid Paper Correction Fluid, lost its distinctiveness after two weeks on mole crickets and appeared white. Some materials like Tech-Pen Ink and Pactra Hi-Glo were particularly easy to score because of their bright and heavily pigmented colors. Other colors such as Creme L'Oreal Nail Accents and Stroblite Paint were less intense and required bright or UV light to be viewed easily.

DISCUSSION

Although the use of many marking materials has been reported in the literature, this is the first extensive, comparative study. Many materials

used by earlier workers have been discontinued or chemically altered. We have attempted to test and compare a large number of materials currently available. However, those using materials reported here should be cautioned that these too may be discontinued or chemically altered without the user's knowledge.

Our ultimate goal in this study was to find marking materials that would be useful for marking a variety of adult insects of different sizes, surface qualities, and habitats for long-term studies. Walker and Wineriter (1981) describe the perfect marking material as one that does not peel, chip or flake, and is durable, non-toxic, easy to apply, quick-drying, light-weight, and available in several easy-to-distinquish colors.

We found that in almost all situations non-water-soluble materials adhered better than water-soluble materials to our test insects. Our results suggest, however, that the nature of the surface being marked affects the durability of the material and whether the results are repeatable. The non-water-soluble materials adhered much better and more consistently to the pubescent pronotum of mole crickets than to the smooth shiny surface of field crickets or the oily surface of flour beetles. However, the more abrasion a material received, as in mole crickets continually tunneling through soil, the more likely the material was to chip, flake, or peel.

The best material for marking insects in long-term studies, according to our tests, is Tech-Pen Ink. Only Tech-Pen Ink stayed on all three species of insects as long as adults are expected to live under field conditions — although the durability of marks on flour beetles varied. We attribute this inconsistency to variation in the oiliness of the beetle pronotum or to slight alteration in the marking conditions or materials. Tech-Pen Ink is available in 11 colors. All are easily distinguishable in daylight, and most are distinguishable by flashlight at night. The inks are not usually available locally and must be ordered from scientific suppliers or the manufacturer in New Jersey (see Appendix). The material comes in a tube without an applicator. Application can be tricky especially when the ink starts to dry and becomes stringy; fresh paint should always be used for best results. For larger insects, this inconvenience can be overcome by using a paint pot similar to one designed by W.D. Hamilton (Walker and Wineriter 1981). For insects the size of flour beetles, application is painstaking; small amounts of the material dry rapidly and the ink must be applied as quickly as possible.

In searching for an optimal marking material, several considerations must be made: the size and habitat of the insect, the nature of the surface to be marked, and the duration of the study. Materials that are water-soluble when wet even though water-resistant or waterproof when dry should be avoided. Non-water-soluble materials will probably work best and a

number will probably work well in short-term studies (4 weeks or less), but the number of durable materials useful for long-term studies, especially on small insects or insects having smooth, shiny or oily surfaces, seems limited. Of eighteen non-water-soluble materials compared in this study, only one, Tech-Pen Ink, was durable on all three insects.

Table 1. Durability[a] of 26 marking materials[b] applied as a dot on each quadrant of the pronotum of 5 individuals of each of three species of insects (N = 20 dots/paint/species).

Non-water-soluble Materials	*G. rubens*	*S. acletus*	*T. castaneum*
1) Tech-Pen Ink			
yellow	A,B (> 7, > 7; 2, > 6)[h]	A (> 18, > 18)	F (< 1, 2)
orange	2A,B (> 5, > 5; > 5, > 5; 1, > 7)[c]	4A,B, B* (> 23, >23; > 18, > 18; > 10, > 10; > 10, > 10; 9, > 15; 6, > 10)[c]	B,2F (5, 29; < 1, 4; 3, 6[i])[c]
white	A (> 7, > 7)	A (> 15, > 15)	F (< 1, 6)
red	B (3, > 8)	? (> 9, > 9)	2B (< 1, 20; 5, > 53)[h]
green	B (< 1, > 5)	A (> 14, > 14)	—
blue	—	—	F (1, 7)
2) Liquid Paper C.F.			
white	B* (< 1, > 6)	A (> 19, > 19; > 18, > 18)[h]	Could not be applied[e]
buff	F (< 1, 4)	A (> 15, > 15)	Could not be applied[e]
green	F (< 1, < 1)	A (> 15, > 15)	Could not be applied[e]
goldenrod	B** (2, > 8)	A,A*(> 15, > 15; > 11, > 11)[h]	Could not be applied[e]
blue	A* (> 7, > 7)	? (> 7, > 7)	Could not be applied[e]
3) Gams Printer's Ink			
dayglo rocket red	B** (2, > 7)	A (> 17, > 17)	F (< 1, 2)
4) Glo-in-the-Dark P.P.	F (< 1, <1)	A (> 17, > 17)	F (< 1, < 1)
5) Pactra 'Namel			
gloss white X-2	B (2, > 5)	A (> 15, > 15)	F (1, 8)
6) Pactra Scale Model F.			
brick red M8	? (> 4, > 4)	A (> 14, > 14)	F (< 1, 2)
7) Lumikwik 566			
daylt.-fluorescent			
red-orange	F (< 1, 3)	A (> 14, > 14)	F (< 1, 1)
8) Pentel White Marker	F (< 1, < 1)	2A*, F(> 11, > 11; 10, > 20; 3, 8)[h]	2F (< 1, < 1; 1, 1[i])
9) Nissen Metal Marker			
red	B (2, > 5)	B (8, > 15)	Could not be applied[e]
10) Pactra Aero Gloss Dope			
swift white 64-1	F (< 1, < 1)	2B** (< 1, > 15; < 1, > 22)[h]	F (< 1, < 1)
11) Naz-Dar Screen Ink			
daylt.-fluorescent			
5593 crimson red	B (2, > 5)	A* (12, > 14)	F (<1, 3)
12) Pactra Hi-Glo			
orange	B (2, > 6)	—	F (≤ 2, 5)[f]
yellow	—	B (9, > 14)	—
13) Stroblite Paint			
yellow-orange	A (> 6, > 6)	B,B*(1, 16; 5, > 12)[h]	F (< 1, 2)
14) Pactra Formula U Poly.			
20073 aviation yellow	? (< 1, > 3)	B (5, 15)	F (< 1, 2)
15) Testors Pla Enamel			
1145 white	? (> 4, > 4)	A (> 12, > 12)	F (<1 , 2)
16) Creme L'Oreal Nail A.			
British redcoat	F (< 1, 2)	A (> 11, > 11)	? (≤ 1, ≥ 6)[g]
17) Sam 100 white	B (2, > 8)	A (> 12, > 12)	F (< 1, 3)
18) Phosphorescent Ink	? (< 1, > 4)	F (< 1, 2)	F (≤ 2, 4)[f]
Water-soluble Materials (waterproof or water resistant when dry)			
19) Stroblite Tempera			
chartreuse VL	B (< 1, > 7)	B** (3, > 14)	F (< 1, < 1)
20) Dupont Latex Enamel			
gloss white, 1800C	F (<1, < 1)	? (4, > 9)	F (< 1, < 1)
21) Speedball Opaque Ink			
red	F (< 1, < 1)	—	F (< 1, < 1)
yellow	—	F (1, 8)	—

	G. rubens		*S. acletus*		*T. castaneum*	
22) Hyplar Acrylic Titanium white H212	F	(< 1, < 1)	F	(≤ 1, ≤ 2)[d]	F	(< 1, < 1)
23) Liquitex Acrylic Titanium white #432	F	(< 1, < 1)	F	(< 1, < 1)	F	(< 1, < 1)
24) Rich Glo Daylight Fluorescent Paint D43 red-orange	F	(< 1, < 1)	F	(< 1, 1)	F	(< 1, < 1)
25) Designer's Gouache 536 zinc white	F	(< 1, < 1)	F	(< 1, < 1)	Would not adhere	
26) Speedball Water-Soluble Fluorescent Block Printing Ink magenta 3428	F	(< 1, < 1)	F	(< 1, < 1)	F	(< 1, < 1)

[a]Durability scoring system.

A = likely to last as long as adult[5] live under field conditions. A paint received an "A" if 100% of the marks stayed on greater than or equal to the following life expectancies: *G. rubens,* 5 weeks; *S. acletus,* 10 weeks, and *T. castaneum,* 20 weeks. The numbers in parentheses are the data (n_1, n_2). n_1 is the number of weeks before the first mark was lost; n_2 is the number of weeks before the median mark was lost. $> n_1$, $> n_2$ means all insects died before any marks were lost. n_1, $> n_2$ means one or more marks were lost but the median mark was not lost before all insects died. Thus, if n_1 is ≥ the life expectancy, the paint is given an "A." (Note that loss of one mark can determine that a marking material does not earn an "A.")

B = some marks likely to be lost, at least in long-term studies. A paint received a "B" if < 100% but > 50% of the marks stayed on for the life expectancy of the insects. Thus, for *G. rubens* $n_1 < 5$, $n_2 \geq 5$.

F = should not be used. A paint received an "F" if < 50% of the marks stayed on for the life expectancy of the insects. For *G. rubens* $n_2 < 5$.

? = insects died too soon to evaluate paint using the above criteria.

If a material stayed on as long as the life expectancy of the insects, i.e. 5 weeks in *G. rubens,* 10 weeks in *S. acletus* and 20 weeks in *T. castaneum,* the material was scored for its adhesive property as well.

A or B - marks rarely peeled, chipped, or flaked. In every week during the life expectancy of the insects, the number of partial marks on living insects was < 10%.

A* or B* - marks sometimes peeled, chipped, or flaked. In at least one week during the life expectancy of the insects, the number of partial marks on living insects exceeded 10% but never 50%.

B** - marks often peeled, chipped, or flaked. In at least one week during the life expectancy of the insect, the number of partial marks on living insects was 50%.

[b]For more information on materials see Appendix.

[c]Since all sets of insects could not be marked on the same day, one set of insects was marked with Tech-Pen Ink, orange, every time insects were marked.

[d]Marks not scored first week, thus $n_1 \leq 1$, $n_2 \leq 2$.

[e]Liquid Paper Correction Fluid and Nissen Metal Marker dried too fast to be applied as very small dots.

[f]Marks were not scored the second week, thus $n_1 \leq 2$ weeks.

[g]Marks were difficult to score (color not very opaque) and were not successfully scored until the second week using a brighter light, thus $n_1 \leq 1$; insects were terminated by mistake before the median mark was lost, thus $n_2 \geq 6$.

[h]More than one set of insects was marked if there was some question about how they were marked or reared.

[i]Data from Walker and Wineriter 1981.

APPENDIX

Listed below is additional information about marking materials that were highly rated on at least one of the test insects. The materials are in the same order as in Table 1.

1) **Tech-Pen Ink,** developed for marking laboratory glassware, 11 colors; Mark-Tex Corp., 161 Coolidge Ave., Englewood, NJ 07631.

2) **Liquid Paper Correction Fluid,** 9 colors, also useful as a background material on which a number can be written with a technical pen (see Walker and Wineriter 1981); Liquid Paper Corp., 9130 Markville Dr., Dallas, TX 75243.

3) **Gams Printer's Ink,** reported as successful marking material on ants (S.D. Porter and B.M. Glancey, pers. comm. 1980), available in 10 daylight-fluorescent colors, appears thick but when applied bleeds gradually, marks near one another merge together; Gams Ink, 1919 W. 2300 South, Salt Lake City, UT 84119.

4) **Glo-in-the-Dark Phosphorescent Paint,** Series P-5100, pale in daylight, glows brightly in dark or under UV light, pigment separates from base readily, must be repeatedly mixed for best results; Conrad-Hanovia, Inc., 100 Chestnut St., Newark, NJ 07105.

5) **Pactra 'Namel,** available in 38 colors (17 flat, 21 gloss), hobby paint, bleeds when

applied, color remains bright over time; Pactra Industries, Hobby Div., 16946 Sherman Way, Suite 300, Van Nuys, CA 90028.

6) **Pactra Scale Model Flats,** available in 24 colors (flat), hobby paint, thin, bleeds when applied, marks become different sizes; Pactra Industries, Hobby Div., 16946 Sherman Way, Suite 300, Van Nuys, CA 90028.

7) **Lumikwik 566,** daylight-fluorescent poster ink, available in 6 colors, thick, easy to apply; Advance Process Supply Co., 400 Noble St., Chicago, IL 60622.

8) **Pentel White Marker,** delivers a fine line of opaque-white, oil base ink that dries quickly, fades over time, must be mixed well for best results; Pentel of America, 1100 Arthur Ave., Elk Grove Village, IL 60007.

9) **Nissen Metal Marker,** ball point (not used, material squeezed through puncture in side of tube),bright opaque ink available in 12 colors; John P.Nissen, Jr., Co., Glenside, PA 19038.

10) **Pactra Aero Gloss Fuel Proof Dope,** hobby paint, available in 25 colors, thin; Pactra Industries, Hobby Div., 16946 Sherman Way, Suite 300, Van Nuys, CA 90028.

11) **Naz-Dar Screen Process Ink,** No. 5500 series silk screen ink, available in 10 daylight-fluorescent colors; Naz-Dar Co., 1087 N. Northbranch, Chicago, IL 60622.

12) **Pactra Hi-Glo,** daylight-fluorescent poster paint available in 6 colors, thick, easy to apply; Pactra Industries, Hobby Div., 16946 Sherman Way, Suite 300, Van Nuys, CA 90028.

13) **Stroblite Daybrite Bulletin Paint,** poster paint, brightest on light surface or on dark surface under UV light, available in at least 4 colors; Stroblite Co., Inc. 10 E. 23rd St., New York, NY 10010.

14) **Pactra Formula U Polyurethane,** hobby paint, available in 18 colors (15 gloss, 3 flat); Pactra Industries, Hobby Div., 16946 Sherman Way, Suite 300, Van Nuys, CA 90028.

15) **Testors Pla Enamel,** hobby paint, available in 100 colors, 6 fluorescent; The Testor Corp., 620 Buckbee St., Rockford, IL 61101.

16) **Creme L'Oreal Nail Accents,** nail polish, thin, not very opaque; Cosmair, Inc., Dist., New York, NY 10036.

17) **Sam 100,** flat poster ink, available in 22 colors, thick, easy to apply; Advance Process Supply Co., 400 N. Noble St., Chicago, IL 60622.

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