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MARKING ADULT COLORADO POTATO BEETLES, *LEPTINOTARSA DECEMLINEATA* (COLEOPTERA: CHRYSOMELIDAE) USING PAPER LABELS

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ABSTRACT

The smooth elytra of adult Colorado potato beetle, *Leptinotarsa decemlineata*, make this insect difficult to make efficiently for long-term field studies. Enamel paint marks fell off rapidly, and after 28 days, 25% of marked beetles had lost all four original marks. Use of small paper labels glued to the elytra after an acetone wash and sanding pretreatment was the most effective method for long term marking of individual beetles. Mortality in labeled laboratory-reared and field-collected beetles did not increase when compared to unmarked beetles.

Adult Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (CPB), are difficult to mark for long-term mark-recapture type field studies. Enamel paints have been used for short-term mark-recapture studies (Bach 1982, Boiteau 1986, Grafius et al. 1985, Williams 1988), but these marks frequently fall off. The smooth elytra and secretion of defensive compounds though the elytra (Daloze et al. 1986, Deroe and Pasteels 1977) reduce adhesion of enamel paints and diminish their potential for longer-term marking. Lack of dense hairy regions limit use of fluorescent dusts. Hare (1983) marked the hind wings with felt tip pens, but this technique requires extensive handling that may damage beetles. Rubidium fluoride was also found unacceptable as a long-term marker as adults excreted the compound within 5 days of ingestion (Voss and Ferro 1985).

Our objectives were to test a paper label system for marking CPB adults for long-term studies. Our first objective was to determine how long enamel paint marks would persist on CPB elytra. The second objective was to determine how long paper labels glued to the elytra would be retained, and if these labels would affect beetle survivorship when compared to unmarked beetles. To determine if the effect of labels on survivorship depended on beetle health, survivorship was compared among marked and unmarked young laboratory-reared beetles, and as a worst-case scenario, stressed marked and unmarked field-collected beetles (beetles where there was high mortality among controls). The system tested has potential for use in field studies of adult Coleoptera, Orthoptera and other insect orders in which there are sufficient areas of cuticle to attach the labels.

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MATERIALS AND METHODS

Two groups of beetles, one from the field and one from the lab, were tested. First generation adult beetles (summer adults) were collected from potato fields at the Michigan State University Montcalm Potato Research Farm in late August 1986 and stored with potato foliage in a cooler (5°C) for six weeks. These beetles were collected just prior to harvest and the conditions were designed to induce beetle dispause in the laboratory. A second set of 1–2 week old adults was obtained from a lab culture at the time of experimentation. This culture had been in the lab for 2.5 years and was originally collected in Antrim County, Michigan. Lab beetles were reared under constant environmental conditions and were believed to be less stressed than the field-collected beetles.

The effectiveness of Testor's® enamel paints as a marking agent was tested by applying four dots of blue paint to each of 40 beetles (two dots per elytra). Four marks were used per beetle because these marks were known in advance to chip off, and redundant marking allowed for some mark loss without total loss of information. The beetles were released in a $1 \times 1 \times 1.5$m cage with six potted potato plants. Mortality of marked beetles was compared to a similar control cage containing 40 unmarked beetles. The cages were sampled weekly to record mortality and number of paint marks remaining on marked beetles. The experiment was concluded after four weeks.

Paper labels were produced on a dot-matrix printer by a computer program that generated all possible 2 character alpha-numeric combinations. Character combinations were edited to reduce confusion between similar marks (e.g. Q and O), and photocopy reduced to produce labels 2.5mm × 1.5 mm. A cyanoacrylate-based glue, Krazy glue®, was used to cement one label per beetle to the surface of the left elytron. Rubink (1988), used a similar method to produce tags to mark native screwworm, *Cochliomyia hominivorax* (Coq.) (Diptera:Calliphoridae), but that method required the use of photographic reduction.

To evaluate the retention of glued-on paper marks on both field and laboratory-reared beetles, treatment units consisting of 10 beetles per treatment were enclosed in a cylindrical wire mesh cage (ca.30cm diam. × ca.60cm tall) (photophase 16h light:8h dark, 20°C). Beetles were free to move about on plant foliage and soil surface or to burrow into the soil. One potato plant was kept in each cage and changed weekly.

To determine the durability of the marks and survivorship of marked beetles, marked field-collected beetles were compared to unmarked controls in greenhouse tests. The experiment consisted of three treatments (each replicated 5 times): (1) a control with no marking, (2) labels glued directly to the elytra with no pretreatment, or (3) labels glued to the elytra after a pretreatment in which the elytra were wiped with acetone and roughened with fine (180 grit) sandpaper. Acetone, which is used regularly in topical insecticide applications, has little or no effect on beetle survivorship (Grafius, pers. obs.) and was used as a wash to clean the elytra prior to applying the glue. Survivorship and label retention were examined every three days for four weeks.

Because the field-collected beetles were collected from high-density populations and chilled for storage prior to treatment, they were expected to represent a worst-case scenario for survivorship. The previous experiment was therefore repeated with lab-reared beetles with two treatments (each replicated four times): (1) an unmarked control, and (2) labeling with the acetone + sanding treatment described above.

Survival distributions of treatments for each experiment were tested for homogeneity (Proc Lifetest, SAS Institute 1985, 529–557). Beetles which burrowed into the soil were removed with plants and counted as censored data on day 12. Beetles still surviving at the conclusion of the experiment were included as right-censored data on day 30.

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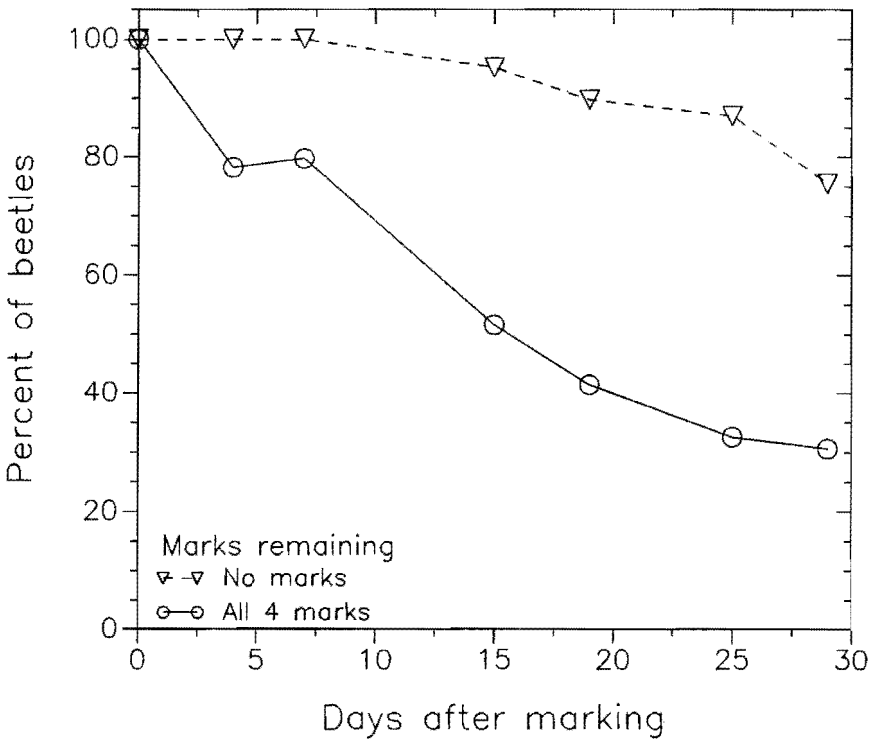

Figure 1. Retention of enamel paint marks by CPB. All beetles were initially marked with 4 dots of blue Testor's model paint.

RESULTS AND DISCUSSION

One week following marking with Testor's enamel model paint dots, 21% of beetles had lost at least one mark (Figure 1), and 50% lost marks after two weeks. All four marks were lost by two of the 40 beetles after 10 days, while 25% lost all marks after 28 days. This procedure is clearly suitable only for short term marking.

For the glued-on paper marks, the acetone + sanding pretreatment significantly increased the adherence of the marks compared to gluing without pretreatment (Figure 2). The mean percentage of beetles still marked at the conclusion of the study was 91.7% (SEM 5.9%) with the acetone-sanding treatment and 38.9% (SEM 16.3%) without pretreatment, (two-sample t-test, $t = 3.026$, $p < 0.05$).

Life table analysis suggest that the null hypothesis of homogeneity of survival probability functions of the three treatments applied to the field-collected beetles (Figure 3) cannot be rejected (Wilcoxon chi-square 1.096, 2 df, $p = 0.58$). Mortality in the lab-reared beetle set was less than 3% (Figure 4), and there was no significant difference between labeled and control treatments.

The method of marking we have developed has great potential for long-term adult CPB and other insects. Large numbers of beetles can be marked individually and relatively efficiently (50–100/hr/person). The marks are small and light enough to be of minimal interference with flight and can be colored to blend or contrast with

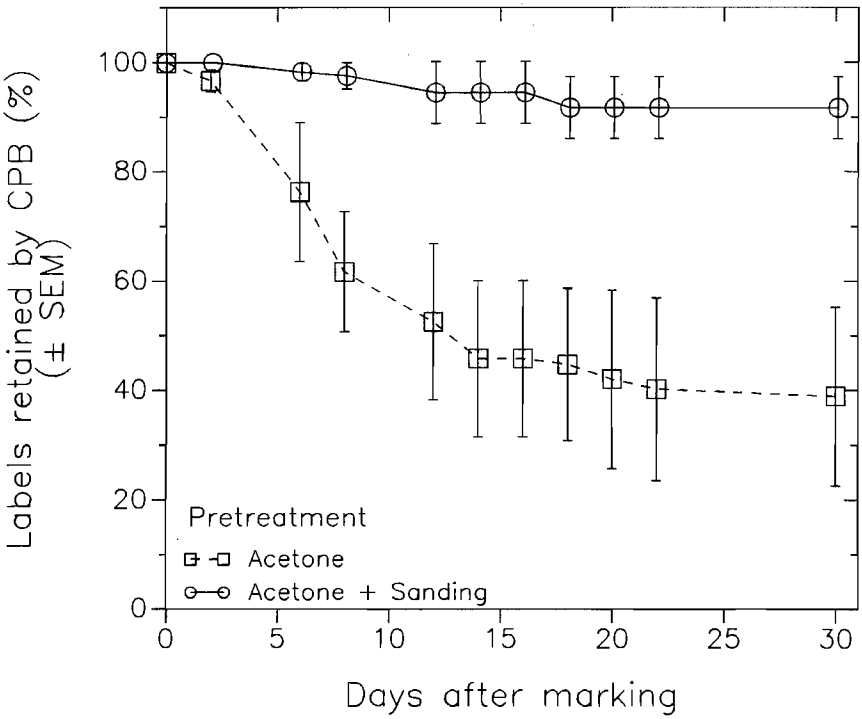

Figure 2. Label retention of field-collected beetles marked with or without an acetone/sanding pretreatment.

the insect's natural coloration. Use of different-colored labels and/or attachment of labels to left or right elytra make it possible to extend the potential number of beetles marked without repetition. Moreover, labels were durable and securely attached, comparable to the most durable materials tested by Wineriter and Walker (1984), and it has been possible to excavate overwintering beetles in the field in late fall and identify them (Caprio 1987). The labels do not appear to affect CPB mortality, even under worst-case conditions.

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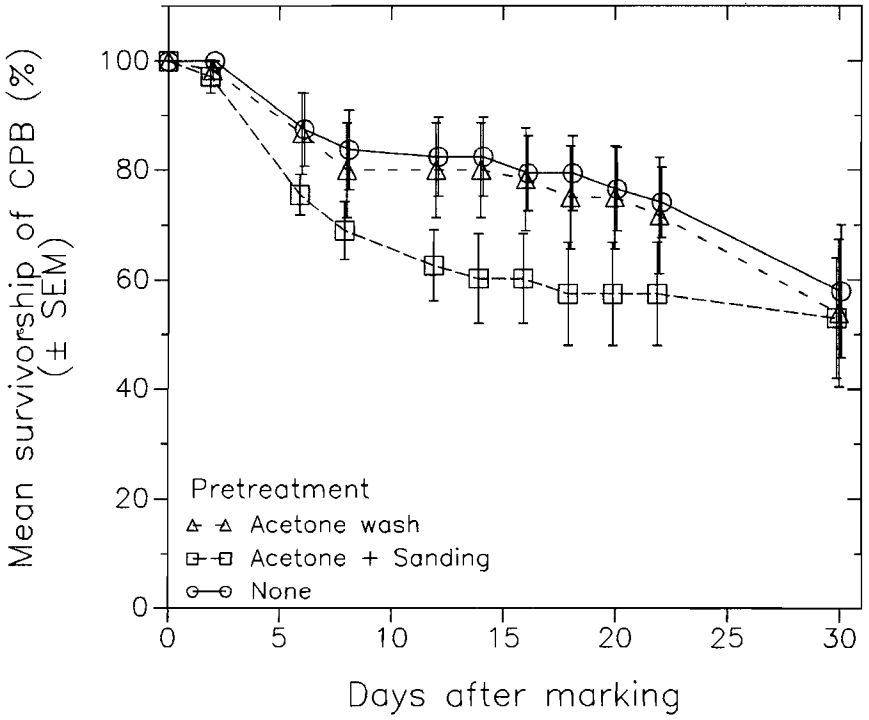

Figure 3. Mean survivorship of control (unmarked) vs. marked field-collected beetles with and without acetone/sanding pretreatment.

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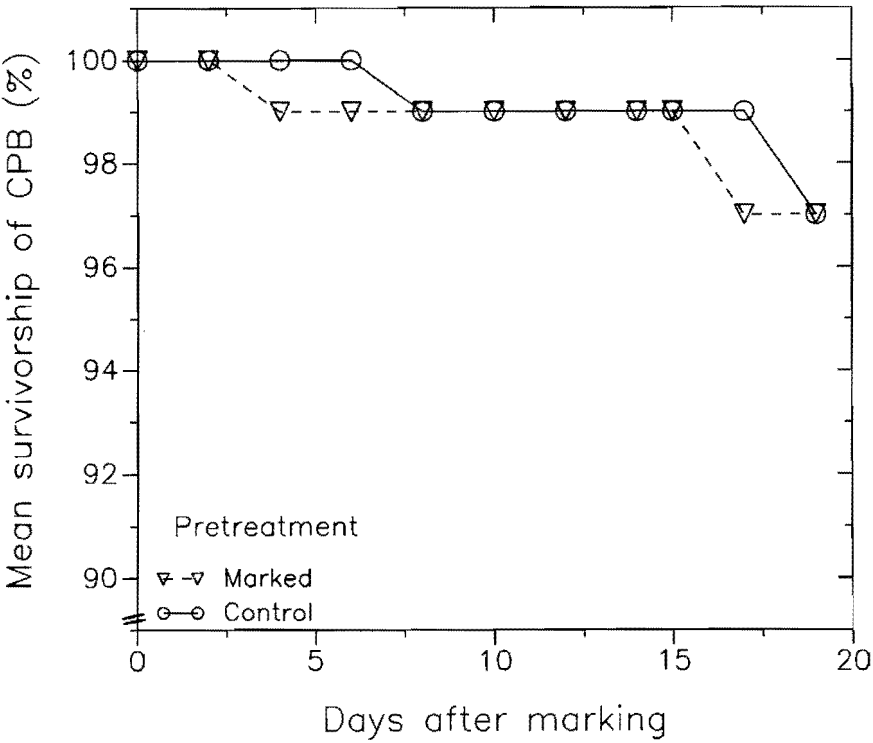

Figure 4. Mean survivorship of marked laboratory-reared beetles with an acetone/sanding pretreatment vs. unmarked beetles. Note: Y-axis scale from 90% to 100%.

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