NOTE

Oil-Soluble Dyes for Marking European Corn Borer (Lepidoptera: Crambidae)[1,2]

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The European corn borer, *Ostrinia nubilalis* (Hübner), is a pest of corn throughout the Corn Belt. It also is the target pest of the first commercially available transgenic corn hybrids. These transformed hybrids contain a modified gene from the bacterium *Bacillus thuringiensis* Berliner that expresses an insecticidal protein (Koziel et al. 1993, Bio/Technol. 11: 194-200). The widespread use of these hybrids has fueled the development of resistance management models and protocols.

A thorough understanding of *O. nubilalis* dispersal and movement is essential to resistance management. Measurements of *O. nubilalis* pre- and post-mating dispersal distances and the identification of the factors affecting dispersal and movement can be used with other biological, genetic, and behavioral measurements to estimate gene-flow and model the development of resistance. Mark-release and recapture techniques often are used to examine dispersal and movement, and a requisite for these techniques is a marking method that does not significantly affect the biology and behavior of the target organism. Various dyes have been incorporated into the larval diet of different lepidopteran species resulting in internally marked adults that suffer little to no adverse effects (Raun 1967, Proc. N. Cent. Branch Entomol. Soc. Am. 22: 162-163; Hendricks et al. 1971, J. Econ. Entomol. 64: 1399-1401; Ostlie et al. 1984, J. Econ. Entomol. 77: 118-120). However, these dyes are not universally suitable across lepidopteran species, so each dye must be tested for the species in question.

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[2]This article reports the results of research only. Mention of a propriety product does not constitute an endorsement or recommendation for its use by USDA.

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Several dyes have been tested and found suitable for internally marking *O. nubilalis* adults and eggs (Raun 1967, Ostlie et al. 1984). Of these dyes only one, Sudan Blue 670, is still commercially available. Therefore, to conduct mark-recapture studies using dyed *O. nubilalis* it is necessary to evaluate currently available dyes for marking the insect. Also, although deemed appropriate for use, Sudan Blue 670 reduces larval survival and delays development (Ostlie et al. 1984). Reducing the dye concentration has been suggested to minimize such adverse effects (Hendricks et al. 1971, Ostlie et al. 1984). Therefore, to test currently available dyes, we examined (1) the effects of a reduced concentration of Sudan Blue 670 on *O. nubilalis* pupation, fecundity, and adult life span, (2) the effects of Sudan Red IV on *O. nubilalis* pupation and adult life span, and (3) the effects of Sudan Orange, Sudan Red 7B, and Neutral Red on *O. nubilalis* fecundity and adult life span.

In 1996 and 1997, dye evaluations were performed using a standard meridic diet (Lewis and Lynch 1969, Iowa State J. Sci. 44: 9-14; Reed et al. 1972, J. Econ. Entomol. 65: 1472-1476). Egg masses were obtained from colonies that were reestablished from feral moths each summer. The 1996 study was conducted at the Entomology Department, University of Nebraska-Lincoln, NE. The 1997 study was conducted at the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA.

In 1996, the experimental design was a randomized complete block with four replications of three treatments: control diet, Sudan Blue 670 (C.I. 61554) and Sudan Red IV (C.I. 26105). Replication was by shelf in a growth chamber. Because Ostlie et al. (1984) reported reduced larval survival and delayed development using 0.6 gm Sudan Blue 670 dye/liter diet, preliminary studies indicated that 0.4 gm dye/liter diet was sufficient to dye the adults. Therefore, 0.4 gm dye/liter diet was used in the subsequent studies. Dye was first incorporated into 100% pure corn oil (1 g dye:10 ml oil) and then incorporated into the diet. The control received corn oil without dye. On 12 April, each of 12 Petri dishes (65 × 150 mm diam.) received 300 ml of diet (4 control, 4 blue, 4 red), 200 neonate larvae (total 2,400), a corrugated cardboard pupation ring, and were placed in the growth chamber (27°C, continuous light). Pupal numbers were recorded 16, 19, and 24 d after infestation (28 April, 1 May, and 6 May, respectively). On 6-10 May, 10 to 11 male and female adults from each replicate-treatment were placed individually in small diet cups and placed in the growth chamber. Those that appeared injured upon transfer were removed (final total: 122 male, 112 female). Moths were given water daily. Adult life span was recorded.

In 1997 the experimental design was completely random with seven replications of a control and four dye treatments. Dyes were Sudan Blue 670 (C.I. 61554), Sudan Red 7B (C.I. 26050), Sudan Orange 220 (C.I. 12055), and Neutral Red (C.I. 50040). Diet was prepared as described above. The larvae were reared as described in Lewis and Lynch (1969) and Reed et al. (1972). The pupation rings were placed in emergence cages on 18 March and adults were allowed to emerge for 2 days. On 20 March the adults were removed and new adults were allowed to emerge overnight. On 21 March the pupation rings were removed from the emergence cages. On 24 March, three male-female pairs/pupation ring were placed in 5.75 × 8.5 cm diam cages (1 pair/cage). The cage was covered with wax paper for oviposition, and the moths were provided water daily. The base and sides were made of brass mesh (approximately 5 grid/cm). The lid was a standard quart-sized canning lid covered with 0.25 in grid hardware cloth with a 0.75 in^2 hole in the center for insertion of moths. The numbers of egg masses were recorded (>5 eggs/mass) 4, 8, and 14 d after

pairing (28 March, 1 April, and 7 April, respectively). The wax paper was replaced after each egg mass count. Adult life span was recorded.

Statistical analysis was conducted using ANOVA to identify treatment effects and means were separated using protected LSD (SAS Institute 1990, User's manual, version 6. SAS Institute, Cary, NC).

Sudan Blue 670, Sudan Red IV, and Sudan Red 7B adequately marked *O. nubilalis* larvae, pupae, and adults. Coloration of individual insects fed on blue diet or red diet varied from light blue to dark blue, and from light red to dark red, respectively. Sudan Blue 670 and Sudan Red 7B also dyed most of the resultant egg masses. Sudan Blue 670 dyed 84% of the egg masses, and Sudan Red 7B dyed 99% of the egg masses. Neutral Red and Sudan Orange did not satisfactorily dye the insects and were eliminated from the analysis.

Means for percent pupation, total number of pupae, and adult life span in 1996 are presented in Table 1. There were no significant differences between treatments (F = 0.22, df = 2, P = 0.80) for total pupal count, indicating there was no effect of the dyes on larval survival. There were no significant differences between treatments for percent pupation (% of total pupae) at 16 or 19 days post egg-hatch (F = 0.15, df = 2, P = 0.86 and F = 1.11, df = 2, P = 0.39, respectively), indicating there were no larval developmental delays associated with the dyes. Also, no significant differences were indicated between treatments for female or male adult life spans (F = 1.31, df = 2, P = 0.27 and F = 2.43, df = 2, P = 0.09, respectively). Most egg masses produced by unmated females were dyed their respective color, although colored egg mass counts were not recorded.

Means for percent egg deposition, total egg mass deposition, and adult life span in 1997 are presented in Table 2. There were no significant differences between treatments (F = 0.39, df = 2, P = 0.68) for total egg mass count, indicating there was no effect of the dyes on fecundity. There were no significant differences between treatments for percent egg mass deposition (% of total egg mass count) 4 days or 8 days post-pairing (F = 2.34, df = 2, P = 0.11 and F = 1.14, df = 2, P = 0.33, respectively), indicating there was no effect of the dyes on the oviposition rate. Again, no significant differences were indicated between treatments for female or male adult life spans (F = 1.93, df = 2, P = 0.16 and F = 0.24, df = 2, P = 0.79, respectively).

Table 1. Mean (±SE) percent pupation (percent of total pupated) at 16 and 19 d after egg-hatch, total pupae, and adult life spans for European corn borer reared on diets with or without dyes

Treatment	% Pupation Day 16	% Pupation Day 19	Total Pupal Count	Adult Life Span (days)	
				Male	Female
Diet alone	48.5 (8.4) a	92.5 (2.8) a	114.3 (15.7) a	18.5 (0.7) a	16.6 (0.7) a
Diet + Sudan blue 670 (C.I. 61554)	45.3 (4.5) a	83.9 (4.0) a	111.3 (9.9) a	17.4 (0.5) a	16.8 (0.7) a
Diet + Sudan red 380 (C.I. 26105)	41.5 (8.9) a	86.8 (3.7) a	102.5 (19.1) a	16.7 (0.6) a	15.5 (0.6) a

Means in a column with the same letter are not significantly different by protected LSD.

Table 2. Mean (±SE) percent egg mass deposition (% of total egg masses) at 4 and 8 d after pairing, total egg masses, and adult life spans (days) for European corn borer reared on diets with or without dyes

Treatment	% Egg Mass Day 4	% Egg Mass Day 8	Total Egg Masses	Adult Life Span	
				Male	Female
Diet alone	58.3 (8.7) a	88.9 (5.3) a	4.5 (1.7) a	14.5 (0.5) a	13.3 (0.5) a
Diet + Sudan blue 670 (C.I. 61554)	80.7 (7.2) a	97.8 (4.3) a	6.4 (1.7) a	14.6 (0.5) a	11.9 (0.5) a
Diet + Sudan red 7B (C.I. 26105)	81.6 (8.5) a	99.3 (5.1) a	6.3 (1.7) a	15.0 (0.5) a	12.1 (0.5) a

Means in a column with the same letter are not significantly different by protected LSD.

Incorporation of the dyes into larval diets at 0.4 gm dye/liter diet satisfactorily colored the adults and had no significant effect on the measured parameters. Although these evaluations focused on marking adult *O. nubilalis,* it appears the dyes also are effective for egg mass marking, and this should be further examined. Considering the above criteria, we believe Sudan Blue 670, Sudan Red IV, and Sudan Red 7B are appropriate for use as internal markers for mass labeling *O. nubilalis* adults.

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