Fluorescent Pigments for Marking Lesser Grain Borers (Coleoptera: Bostrichidae)

A. K. DOWDY AND W. H. McGAUGHEY

U.S. Grain Marketing Research Laboratory, USDA–ARS, 1515 College Avenue, Manhattan, Kansas 66502

J. Econ. Entomol. 85(2): 567–569 (1992)

ABSTRACT Fluorescent dyed melamine copolymer resins were evaluated to determine their effectiveness for marking adult *Rhyzopertha dominica* to be used in release–recapture experiments. Pigments were retained very well, and marked individuals were easily identified under longwave ultraviolet light up to 21 d after treatment. Little pigment was transferred between individuals during mating or other contact within a bulk of wheat. Treatment with fluorescent pigment did not reduce fecundity or prevent flight activity.

KEY WORDS Insecta, fluorescent pigment, marking, *Rhyzopertha dominica*

IDENTIFICATION OF individual insects or groups of insects within a population is useful for estimating population size or studying movement within an environment. A simple method for marking lesser grain borer, *Rhyzopertha dominica* (Fab.), is needed to study the extent of directional movement from sources of infestations. It is important that the identifying mark not affect the behavior of the individual or the response of other insects to the marked insect.

The lesser grain borer feeds within grain kernels and is a severe pest of stored grain. Because of its small size (3 by 1 mm) and habits, a suitable mark must be lightweight and attached sufficiently so that the insect does not lose the mark or cause significant contamination to the grain or other insects as it crawls among or within grain kernels.

A number of insect marking techniques are discussed by Southwood (1978), including mutilation of the organism or the use of materials such as rare elements, radioactive isotopes, and fluorescent dyes dissolved in acetone or mixed with lacquer. Marking by mutilation is difficult because of the small size of the lesser grain borer. Similarly, using paint or lacquers is ineffective for mass marking of beetles because droplets tend to glue elytra closed and inhibit leg and antennal movement (A.K.D., personal observation). The use of rare elements or radioactive isotopes is also undesirable because of the additional cost of application and detection equipment, and waste disposal.

Stern & Mueller (1968) reported successful results with direct application of Helecon luminescent pigments to a number of insect species. The adherence qualities of the pigments appeared to be related to morphological characteristics of a species. Insects with dense or long setae or well-pronounced sutures retained the pigments best. Moth & Barker (1975) documented that a *Drosophila* sp. marked with Helecon pigments was detected after 4 wk with no reduction in viability. Fluorescent pigments mixed with sand or corncob grit were successfully used for marking boll weevils, *Anthonomus grandis* Boheman, for up to 12 d and did not cause early mortality (Reinecke 1990). However, because the pigments did come off in a short time, it is important to determine if this material would be retained by lesser grain borers for more than a few days in a bulk of grain.

The three objectives of this study were to determine (1) if lesser grain borer adults could be successfully marked with fluorescent pigments and whether marked insects contaminated unmarked insects; (2) if the pigments are successfully retained by adults moving and feeding in wheat, *Triticum aestivum* L.; and (3) if the fluorescent pigments prevent insect movement, particularly flight.

Materials and Methods

Fluorescent pigments used in the experiments were dyed melamine copolymer resins with an average particle size of 4.5–5.0 μm (Day-Glo Color Corp., Cleveland, Ohio). Colors used were aurora pink (A-11), blaze orange (A-15), and Saturn yellow (A-17-N).

The first experiment was designed to determine the optimum length of exposure to pigments necessary to mark adult lesser grain borers adequately with the three colors of pigment. Nine replicates of five adults were placed in a 100-ml test tube to which 0.5 ml of pigment was

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

added and gently shaken for ≈5 s. The beetles remained in the test tube for 5, 15, or 30 min (n = 15 for each time period) and then were emptied on filter papers and allowed to crawl out of the excess pigment. After 30 min, the beetles were placed individually in 25-ml vials that each contained one kernel of wheat. Vials were covered with fine mesh wire lids, and held at 26.7°C and 60 ± 5% relative humidity. The beetles were microscopically examined daily for 11 d using longwave (3660 Å) ultraviolet (UV) light to detect the presence of adhering fluorescent pigment. Percent mortality was determined when the experiment was terminated.

A second experiment was conducted to determine how well the fluorescent pigments adhered to adult lesser grain borers that fed on several kernels of wheat. Three replicates of 15 beetles were treated with each fluorescent pigment as previously described except that all were treated for 15 min. The marked insects were transferred individually to 25-ml vials containing 10 kernels of wheat and held at 26.7°C and 60 ± 5% relative humidity for 21 d. At 3-d intervals, three individuals of each color were microscopically examined to detect the presence of fluorescent pigment as previously described, then returned to their respective vials. Kernels of wheat were also examined to detect any transfer of pigment from the insects. Percent mortality of the beetles was calculated at the end of the experiment.

A third experiment determined whether fluorescent pigment from marked individuals would be transferred to unmarked insects that inhabited the same bulk of grain. Adult beetles were treated with fluorescent pigment as described in the second experiment. The treated insects were then placed in 0.9-liter jars containing 200 g of whole wheat for 24 h. This process removed excess pigment from the marked insects before they were transferred to another jar that contained 200 g of whole wheat and a known number of unmarked individuals. Marked and unmarked beetles were tested in groups of 50 or 100 in which the percentage of marked individuals was 20, 30, or 50%. Overall, 30 replicates were examined. After 72 h, the insects were sieved from the wheat and the percent of apparently marked and unmarked individuals was determined in a blind test. Observed data were compared to the actual number treated using a t test.

A fourth experiment examined the potential for transferring pigment from one individual to another during mating and determined if the presence of the pigment reduced fecundity. Adult virgin males and females (n = 30 pairs) were treated with yellow and pink fluorescent pigment, respectively, and excess pigment removed as described in experiment 3. Pairs of marked beetles were then placed in 25-ml vials with 5 g of cleaned whole wheat. The pairs were left for 7 d at 26.7°C and 60 ± 5% RH and then examined microscopically using UV light to determine if different colors of fluorescent pigment had been transferred between individuals. After examination, the pairs were returned to the vial for 7 d more and then removed. The grain was incubated for an additional 8 wk and progeny were counted to compare fecundity of the marked females with a control group of 20 unmarked females.

A fifth and final experiment in the field determined if lesser grain borers were capable of flight after being marked with fluorescent pigment. Adult beetles were marked and excess pigment removed as previously described in experiment 3. The marked insects were released at two points 12.5 m N and S of a tub containing 38 kg of wheat ('Victory', 11.5% moisture, 755 g/liter). A shallow pan containing 1 kg of wheat that had been previously infested with *R. dominica* and contained damaged kernels, dead insects, and frass was pushed into the surface of the wheat in the tub to increase the attractiveness for released beetles (unpublished data). To detect insect movement, plastic picnic plates (22.5 cm diameter) coated with Sticky Stuff (Olson Products, Medina, Ohio) were mounted vertically on wooden stakes 15–20 cm aboveground and positioned in concentric circles around the tub of wheat at distances of 0, 5, and 10 m with 8, 8, and 12 equally spaced stakes per circle, respectively. The plates were checked 1, 5, and 7 d after release and the grain was sieved 5 and 7 d after release. Average daily temperature during the experiment was 22.9 ± 0.2°C, wind speeds averaged 5.0 ± 0.1 km/h from the southeast, and precipitation totaled 6.0 cm.

Results and Discussion

All adult lesser grain borers held on individual wheat kernels in experiment 1 retained fluorescence regardless of the pigment used or the length of exposure to the bulk pigment. In the second experiment, when treated beetles were held on 10 kernels of wheat for up to 21 d, the pink and orange pigments were retained by all 30 insects for the duration of the experiment. The yellow pigment was retained by all 15 individuals for 7 d but was lost by four over the next 14 d. Some individuals still retained pigment 29 d after treatment.

Marking adult lesser grain borers with fluorescent pigment did not appear to increase mortality relative to natural mortality of those not treated. In experiment 1, mortality was 4% for the treated beetles compared with 0% for those not treated. In the second experiment, mortality was 30% for those insects treated with fluorescent pigment compared with 35% for those left untreated. Higher mortality in the second experiment may

Table 1. Accuracy of identification of marked *R. dominica* in 200-g samples of wheat containing different ratios of marked and unmarked insects

Replicates	No./replicate	% marked		Calculated *t* value
		Actual	Observed	
4	50	50	51.9 ± 5.7	1.069
6	50	30	32.6 ± 2.1	3.189*
5	50	20	22.0 ± 4.3	1.675
5	100	50	53.5 ± 4.6	2.100
5	100	30	33.4 ± 5.0	1.873
5	100	20	20.8 ± 1.5	1.410

*, Indicates significance at $P \leq 0.05$.

have resulted from damage to insects that had to be physically removed from wheat kernels.

In experiment 3, fluorescent pigment was transferred from marked to some unmarked beetles in the same bulk of grain, but marked individuals retained sufficient amounts of pigment to be distinguished from the originally unmarked individuals (Table 1). In the previous experiments, insects originally marked had fluorescent pigment adhering between sternal plates but this was not observed on unmarked beetles. The observed percentage ($\bar{x}$ ± SEM) of marked lesser grain borers increased by only 2.2 ± 1.5% when a total of 50 insects were in the container (t = 3.075, $P \leq 0.05$, df = 14) and by 2.5 ± 1.8% with 100 insects (t = 3.001, $P \leq 0.05$, df = 14).

Little fluorescent pigment was transferred from one insect to another that could be attributed to mating in experiment 4. Males occasionally had colored pigment from the female on antennae, legs, and the ventral surface that may have been obtained during mating. Females exhibited fluorescent pigment from males on mouthparts and legs but rarely on the back or in the area of the genital opening as was expected if transfer had occurred during mating. Although transfer did occur between males and females, this did not affect our ability to separate marked and unmarked beetles accurately. The number of offspring from females treated with fluorescent pigment averaged 25.4 ± 2.7 compared with 26.2 ± 2.9 per female for the control group (t = 0.421, $P > 0.05$, df = 39).

Fluorescent pigment was readily transferred to wheat kernels and retained particularly well in the crevice region and brush. The pigment was not removed by blowing compressed air through the grain nor by washing the grain with water alone or detergent in water. Although the pigments contain no hazardous ingredients (oral $LD_{50} > 16$ g/kg, acute inhalation $LC_{50} > 4$ mg/4 h [Anonymous 1986]), they would probably be undesirable for use on grain that may enter marketing channels.

In the field experiment, five marked lesser grain borers were recovered on sticky plates 12.5 m from the release site the day after the release was made. After 7 d, a total of 148 lesser grain borers were recovered up to 12.5 m from the release site. An additional 24 marked lesser grain borers were also recovered from the grain. These results indicate that lesser grain borers marked with fluorescent pigments were not prevented from flying to a host food source. This experiment did not indicate whether flight may be reduced by marking. However, using the same marking material on western corn rootworm, *Diabrotica virgifera virgifera* LeConte, Naranjo (1990) reported no effect on flight activity under laboratory conditions. Although rain occurred in the study area, all beetles that were recovered still retained enough fluorescent pigment to be identified.

In conclusion, dyed melamine copolymer resins were effective in marking adult lesser grain borers with minimal transfer to other individuals, either by incidental contact or during mating. Although some pigment was lost by lesser grain borers while crawling among kernels of wheat, sufficient amounts remained to identify marked insects. Treatment of adult lesser grain borers with fluorescent pigments did not reduce fecundity or prevent flight activity.

Acknowledgment

We thank W. Blodgett and R. Hammel for their assistance in the laboratory and field.

References Cited

Anonymous. 1986. Material safety data sheet: "A" series pigments. Day-Glo Color, Cleveland, Ohio.

Moth, J. J. & S. F. Barker. 1975. Micronized fluorescent dusts for marking *Drosophila* adults. J. Nat. Hist. 9: 393–396.

Naranjo, S. E. 1990. Influence of two mass-marking techniques on survival and flight behavior of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83: 1360–1364.

Reinecke, J. P. 1990. A rapid and controllable technique for surface labeling boll weevils with fluorescent pigments. Southwest. Entomol. 15: 309–316.

Southwood, T.R.E. 1978. Ecological methods, 2nd ed. Wiley, New York.

Stern, V. M. & A. Mueller. 1968. Techniques of marking insects with micronized fluorescent dust with especial emphasis on marking millions of *Lygus hesperus* for dispersal studies. J. Econ. Entomol. 61: 1232–1237.

Received for publication 8 January 1991; accepted 28 October 1991.