A Simple, Effective, and Low-Cost Method For Mass Marking Adult Western Corn Rootworms (Coleoptera: Chrysomelidae)[1]

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ABSTRACT The feasibility of mass marking western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, beetles with fluorescent powder was studied in field cages and with release-recapture. In field cage studies, no significant ($P > 0.05$) relationship was found between beetles marked with red or green fluorescent powder and beetle mortality. Red marks on beetles were visible with the unaided eye for at least 10 days after marking; however, a binocular microscope was needed to detect green marks on 50% of the individuals after only 4 days. Marking females with flourescent powder did not significantly affect ovarian development. From two releases of red-marked beetles made in the center of a 16 × 40.5-m cornfield, 6.5% of 3,909 and 8.4% of 1,873 adults were recaptured during trapping periods of 10 to 13 days by semiochemical-treated and blank sticky traps placed around the perimeter or within the field. Ovarian development of the marked females recaptured with the traps was not significantly different from that of unmarked beetles caught on the same traps during the same time period.

KEY WORDS *Diabrotica virgifera virgifera,* mark-recapture, western corn rootworm, Coleoptera, Chrysomelidae, fluorescent powder.

Intra- and inter-field movement of western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, adults have not been studied in detail. One method of studying insect movement is to release and recapture marked insects; however, marking must not alter behavior or affect survival. In addition, the marks should be durable, easily recognized, and quickly applied (Gangwere et al. 1964, Southwood 1978).

A large number of mass-marking techniques have been developed for insects in general (Southwood 1978), but techniques for marking corn rootworms have generally proved to be labor intensive or of limited success. Steffey (1979) marked southern corn rootworm, *D. undecimpunctata howardi* Barber, beetles with Day-Glo® fluorescent spray paint and reported that the technique did not greatly affect the longevity or behavior of adults. He then used this method to mark 50,000 individuals of a mixed population of western and northern corn rootworms (NCR), *D. barberi* Smith and Lawrence. He released them in a cornfield but recaptured only 106 NCR and 34 WCR beetles using yellow sticky traps during a 10 day trapping period (total return of 0.28%). Steffey did not account for the low

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recapture rate nor did he report on the effect of marking on WCR and NCR survival. Wisniewski (1984) marked individual WCR with Testor's® model paint to study effects of wind speed and direction on movement of males toward a pheromone source. He reported that marking did not affect the flight ability of beetles in tests conducted in the laboratory. In the field, 2.4% of 12,741 marked individuals were recaptured by Pherocon AM traps baited with Hercon® Luretape™ pheromone dispensers during 36 days of trapping in 1982 and 1983. Haddock (1984) marked individual NCR with enamel paint and reported that the percentage mortality for marked and unmarked beetles in laboratory studies was similar at 4 and 12 days after marking. He also studied the short-range movement of NCR by releasing 886 and 1,295 marked beetles on 18 and 24 August, 1983, respectively, 10 m from two corn plots, one 6 × 6 m and the other 18 × 2 m. All NCR seen in the plots were collected the following day. For the first release, 2.4% of the marked beetles were recaptured; for the second, 5.3%.

Here we describe a simple, effective, and low-cost technique for mass marking WCR with fluorescent powder and evaluate the effects of marking on adult flight behavior, beetle mortality, and female ovarian development. Flourescent powders have been used previously to mark different species of arthropods including boll weevils (Taft and Agee 1962), face flies (Turner and Gerhardt 1965), bees (Frankie 1973), and spider mites (Brandenburg and Kennedy 1982).

Materials and Methods

WCR beetles were collected in cornfields in Champaign, Illinois, in July and August 1988 by shaking adults off of corn (*Zea mys* L.) silks, tassels, and leaves into a funnel mounted on the lid of a 2-liter glass canning jar. Collections were made during the morning hours (0800 - 1100) when beetle activity was minimal. The funnel was replaced with a screen top, and the adults were brought to the laboratory. There they were provided with silk, tassel, or ear tissue depending on the period of the test and the availability of food material. Water was provided by inverting a 30-ml plastic cup covered with a Handiwipe® kitchen cloth over the screen top of the jar. Immediately before marking (at ca. 1600 h), adults were temporarily immobilized (for counting and transferring) by chilling them for 5 min at 5°C. Other means of anesthetization (e.g., ether or carbon dioxide) were not used because they are known to affect insect behavior (Edwards and Patton 1965, Andow and Wetzler 1982). Individuals were aspirated and transferred to 1-liter, covered plastic containers; 500 beetles were placed in each container. Fluorescent powder (0.7 g red no. 335 or green no. 311; Radiant Color, Richmond, California) was added to each container, and the beetles were gently shaken in the powder for 10 sec. Marked beetles were immediately transferred to 1-liter glass canning jars with screen lids containing food and water. Beetles remained in the laboratory overnight (at 24°C) under a 14:10 photoperiod (L:D).

Marked beetles were used in two separate studies. A cage study was conducted in the field to examine the effect of marking on mortality and ovarian development and to determine the durability of marks. A release and recapture study was undertaken to determine the durability of marks and the effect of marking on ovarian development under actual field conditions.

Cage Studies. At ca. 0800 on the morning following marking, beetles were transferred to 30 × 30 × 30 cm screened aluminum cages containing silk, corn ears,

tassel, and water (provided by a saturated piece of cotton in the bottom of an open petri dish). Beetles marked with each color of powder (red, green, and an unmarked control) were placed in separate cages, and these were placed in a cornfield beneath the plant canopy. Each morning, dead beetles were removed from the cages. Every 3-4 days about 10-15 individuals were randomly aspirated from each cage and brought to the laboratory where the visibility of the fluorescent powder was noted with the unaided eye and under a binocular microscope (60x magnification). Tests with red-marked beetles were conducted on 6-16 July, 19-31 July, and 20 August - 1 September 1988. Green-marked beetles were tested only on 6 - 16 July. Two hundred beetles per cage were used for the first and second tests and 100 for the third test. In the second and third tests, those females brought to the laboratory were also examined for ovarian development using a modified method of Cinereski and Chiang (1968). Each female was categorized according to reproductive status as preovipositional stage 1, ovaries undeveloped and a large amount of fat body present; preovipositional stage 2, some eggs beginning to develop; preovipositional stage 3, many immature (unchlorionated) eggs present; ovipositional stage 4, fully developed and chorionated eggs present, beginning of oviposition; and postovipositional stage 5, spent ovaries, all eggs depleted.

Release-Recapture Studies. Two releases were made during the summer of 1988, one on 6 July with 3,909 red-marked WCR and another on 18 August with 1,873 WCR. Beetles were released at ca. 0800 the morning following marking in the center of a cornfield (16 m × 40.5 m) adjacent to the collection site. Marked beetles were recaptured with 540-ml yellow plastic cups (no. P-16; Solo Cup Company, Urbana, Illinois) coated with an adhesive (Tangle-Trap®) and inverted over wooden stakes 1 m in length (Levine and Metcalf 1988). Sixteen semiochemical-treated and 16 untreated traps were placed alternately 4.6 m apart around the perimeter of the field and monitored for 10-13 days following each release.

Semiochemical-treated cups had 100 mg of attractant (equal parts by weight of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde; Aldrich Chemical Company, Milwaukee, Wisconsin) applied to a dental wick placed on top of each inverted cup (Lampman and Metcalf 1987). An additional 18 untreated traps were positioned 4.6 m apart along two intersecting transects through the center of the field (14 traps east to west and 4 traps north to south). Traps were monitored daily, and marked WCR were removed. Unmarked beetles were also brought back to the laboratory for microscopic examination to ensure that no marked beetles were missed. The reproductive status of all marked beetles and of a representative sample of at least 10 unmarked females per day was determined following the method described previously. Wicks containing attractants were changed weekly.

Data analysis. Mortality data obtained from the field cage studies for marked and unmarked beetles were compared with the chi-square test (Little and Hills 1975). The same procedure was used to test for differences in frequency of individuals in each reproductive status class in both field cage and release-recapture studies.

Results and Discussion

Cage Studies. Analysis of the data for the first field cage test indicated that there was no significant ($P > 0.05$) relationship between marking with red or green fluorescent powder and beetle mortality. In addition, when data from the

three tests were pooled, no significant relationship was observed between beetles marked with red fluorescent powder and mortality. Percentage mortality for adults marked with red fluorescent powder was $46.2 \pm 15.6\%$ ($\bar{X} \pm SE$, $n = 3$ cages); mortality for unmarked beetles was $42.8 \pm 22.7\%$.

Green fluorescent powder proved less durable than red. In the first cage test, markings on 5 of 10 green-marked individuals were not visible with the unaided eye 4 days after marking; however, all were clearly marked when the beetles were examined under a binocular microscope. Markings on 10 red-marked individuals were easily visible with the unaided eye 4 days after marking. At the end of this test (11 days after marking), markings on 37 of 43 red-marked individuals were visible with the unaided eye whereas markings on only 2 of 12 green-marked individuals were visible even under the microscope. For this reason, green powder was discontinued for the second and third field cage tests. At the termination of cage tests (11-13 days after marking), red markings on 85.4% of the surviving beetles ($n = 192$) were visible with the unaided eye; markings on the remaining 28 individuals were detectable with a binocular microscope. Markings were especially evident on those portions of the body where beetles could not clean themselves (e.g., intersegmental membranes, leg joints).

Ovarian development of red-marked and unmarked beetles was examined at three dates during each of the second and third field cage tests. The marking technique had no significant effect on ovarian ratings on any sample date. Ovaries of 10, 14, and 20 marked beetles in the second test were in stages 1.7 ± 0.33 ($\bar{x} \pm SE$), 2.0 ± 0.36, and 2.0 ± 0.27 on the three sampling dates of 27 July, 29 July, and 1 August, respectively. Ovaries of 10, 16, and 15 unmarked females on the same sampling dates were in stages 2.1 ± 0.31, 2.3 ± 0.28, and 1.9 ± 0.32. In the third test, approximately one month later, 10, 10, and 15 marked females had ovaries in stages 4.0 ± 0, 3.9 ± 0.18, and 3.9 ± 0.09; 10, 10, and 14 unmarked females had ovaries in stages 4.1 ± 0.10, 3.6 ± 0.16, and 3.9 ± 0.07 on 24 August, 29 August, and 1 September, respectively. Mass marking of WCR using the procedure described here appears to have no significant impact on ovarian development.

Release-Recapture Studies. Approximately 6.5% of the marked individuals from the first release and 8.4% from the second were recovered during 10 and 13 days of trapping, respectively, from all semichemical-treated and untreated traps placed around the perimeter and from all untreated traps placed in the cornfield (Table 1). Most of the recoveries occurred within the first few days after release. Percentage recovery in our study was much higher than that in previous studies with WCR (Steffey 1979, Wisniewski 1984). Only five marked individuals were detected by microscopic examination of all apparently unmarked beetles caught in the traps during the two releases. Within 2 h after beetle release, most marked individuals were crawling up on corn plants and taking flight. We observed no abnormality in the flight behavior of the marked individuals in the field. This observation is in agreement with Naranjo (unpubl.) who found that marking WCR with fluorescent powder (Day-Glo®) had no effect on trivial or sustained flight performance of either males or females in tethered-flight assays. In our two studies, a total of only 29 beetles was found dead at the release point following both releases.

Table 1. Number of marked WCR recaptured by 16 traps baited with semiochemical (equal parts by weight of 1, 2, 4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde; TIC) and 16 unbaited (blank) traps placed around the perimeter (border) and by 18 blank traps placed within a cornfield, Champaign, Illinois, summer 1988.

Days	July 6 release*				August 18 release*			
after release	TIC border	Blank border	Blank inside	Cumul. % recaptured	TIC border	Blank border	Blank inside	Cumul. % recaptured
1	148	10	12	4.35	14	0	16	1.60
2	10	2	7	4.84	6	3	17	2.99
3	2	1	14	5.27	22	1	23	5.45
4	0	2	4	5.42	10	0	9	6.46
5	0	1	11	5.73	7	3	2	7.10
6	0	1	9	5.99	1	0	0	7.15
7†	0	1	7	6.19	0	0	4	7.36
8	0	2	4	6.34	8	0	1	7.84
9	1	1	1	6.42	7	1	0	8.28
10	0	0	1	6.45	0	0	1	8.33
11	-‡	-	-	-	0	1	0	8.38
12	-	-	-	-	0	0	0	8.38
13	-	-	-	-	1	0	0	8.43
Total	161	21	70		76	9	73	

* 3,909 and 1,873 WCR were released on 6 July and 18 August, respectively.
† Wicks replaced.
‡ Trapping period was terminated.

During the first week after the first release in which the majority (96%) of recaptures occurred, all of the marked (n=17) and unmarked (n=17) females caught in semichemical traps were at the first stage of ovarian development, i.e., their ovaries were not developed. In the second release, ovarian development of the marked females recaptured in the semiochemical traps during the first and second weeks ($\bar{x}$ ovarian rating $\pm$ SE = 4.4 ± 0.11, n=37 during the first week, and 4.8 ± 0.09, n=18 during the second week) were not significantly different from ovarian development of unmarked females caught in the traps during the same period (4.4 ± 0.19, n=21 during the first week and 4.6 ± 0.09, n=32 during the second week).

Marking WCR beetles with fluorescent powder is rapid, inexpensive, and requires little labor. Marks are durable and have no evident effect on WCR movement, adult survival, and female ovarian development. This technique should be useful in further study of the intra- and inter-field movement of WCR beetles.

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