Marking Adult Diamondback Moth, *Plutella xylostella* (L.), and Estimation of Adult Survival Rate and Population Density in a Cabbage Field¹

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Nine non-commercial colored aerosol dyes were screened in the laboratory to determine their suitability for marking the adults of *Plutella xylostella* in the field. The satisfactory dyes were Solar Pure Blue, Acid B. M. Green, Solar R. B. and Acid Scarlet B. A. These dye marks were easily recognized on the moths. None of the dyes tested did not affect the longevity of the moths and the dyes remained better on the body than on the wings when water was applied. Solar Pure Blue and Solar R. B. marked moths did not differ significantly from unmarked moths in longevity, fertility and the number of mating pairs. A method of marking *P. xylostella* adults in the field was described. The survival rate of moths in a summer cabbage plot, estimated using JACKSON's (1939) 'positive method' was 0.67 per day.

INTRODUCTION

HARCOURT (1963) suggested that the mortality of adults is the critical or 'key' factor determining population trends of *Plutella xylostella* in cabbage fields. To date there is little information on the estimation of adult survival rates of this species under field conditions. Some methods (e.g. pheromone and light traps) have been used for estimating adult populations in the field, but these methods provide only relative density when the trapping efficiency is not known. It is also difficult to obtain a reliable estimate of the number of adults by direct counting on plant because of their relative mobility.

The mark-recapture method has been used to estimate the survival rate and population density of many insects, such as the green leafhopper, *Nephotettix cincticeps* (NAKAMURA et al., 1967), the melon fly, *Dacus cucurbitae* (ITÔ et al., 1974) and the diamondback moth, *Plutella xylostella* (SHIRAI and MURATA, 1987). In this study, we used JACKSON's (1939) 'positive method' which has been found useful for open populations of insects (RICHARDS and WALOFF, 1954; ITÔ et al., 1974).

The objectives of this study were to evaluate suitable dyes which could be used for mark-recapture studies of adult *P. xylostella* in the field, and to develop a method for

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marking the adult moths in the field to determine their survival rate and population density.

MATERIALS AND METHODS

A. Laboratory tests

Insects and marking procedures: The *P. xylostella* adults used for marking tests were obtained from a laboratory culture reared by the method of NOPPUN et al. (1983). All the experiments were done under the condition of $25 \pm 1^\circ\text{C}$, 70–80% relative humidity and 16 hr photophase.

One day old adult moths were confined in a plastic cup (6.0×9.0 cm), and were anaesthetized with carbon dioxide. For marking, the moths were transferred to a 200 ml (101×44 mm) vinyl chloride cup which was covered on the top with a 28-mesh wire screen. Marking of the moths with various color dyes (Sumitomo Chemical Co. Ltd.; Table 1) was done by spraying each dye on the moths through the mesh screen for ca 3 to 5 s, with the nozzle of the can held ca 20 to 25 cm from cup. In preliminary experiments we found that overspraying caused some mortality of the moths. The marked moths were then transferred to another plastic cup for subsequent tests.

Test 1: Thirty adults were used for each color test to determine the longevity of marked and unmarked adults. The marked insects were kept in a plastic cup (6.0×9.0 cm) to record daily mortality. 'Control' (unmarked) moths were anaesthetized with carbon dioxide only. To determine the retention of marks against removal by water, dead moths which had been marked were laid on a piece of white filter paper (Toyo®), and water droplets were dropped on them. The wash-off of the marks was detected as colored-stains on the filter paper.

Test 2: Two colors, Solar Pure Blue and Solar R. B., which were found to be promising markers in test 1, were chosen to determine their effect on adult longevity and fertility of the moths. About one day old but mated pairs of moths were marked with each of the colors and a pair was confined in a plastic cup (6.0×9.0 cm). Fifteen pairs

Table 1. Longevity and degree of visibility of laboratory-bred *P. xylostella* adults treated with colored spray dyes

Color	Longevity (Days) ^a (mean $\pm$ S.E.)	Visibility ^b rating
Acid Blue Black	4.47 $\pm$ 0.41	+
Solar Pure Yellow	5.53 $\pm$ 0.47	—
Solar R.B.	5.53 $\pm$ 0.46	++
Acid Violet SB	5.60 $\pm$ 0.57	+
Tartrazine NSC	5.80 $\pm$ 0.47	—
Solar Orange	5.90 $\pm$ 0.52	—
Solar Pure Blue	5.97 $\pm$ 0.57	++
Acid Scarlet B.A.	6.70 $\pm$ 0.50	++
Acid B.M. Green	6.77 $\pm$ 0.72	++
Control (unmarked)	5.67 $\pm$ 0.61	

^a No significant difference was obtained among treatment means ($N=30$) at $p>0.05$ by Anova test.

^b Scale: ++ very clear, + moderately clear, — not clear.

were used for each color and 'control', the latter moths being anaesthetized with carbon dioxide only. The longevity and fertility of females and longevity of males were determined until death.

Test 3: Sexing of the moths was done in the larval stage based on the characteristic pale whitish mark on the dorsum of the eighth abdominal segment of male third instar larvae, and larvae of each sex were then bred separately to avoid post-emergence mating. Adults that subsequently emerged were marked with the dyes used in test 2. Ten marked males and ten marked females were then confined in a plastic cup (6.0 × 9.0 cm). The number of pairs in copula was recorded every 15 min after which the mating pairs were removed from the cup. As control, ten pairs of unmarked moths were similarly confined in a cup. The test was conducted for 1 hr and there were three replications for each dye and control.

B. Field test. This experiment was carried out in a cabbage field (20 × 20 m) at the University Farm at Togo, Aichi Prefecture, in June 1985. The field was arbitrarily divided into four quadrats and each quadrat consisted of 10 rows of cabbage with ca 20 plants in each row. In each quadrat, all the cabbage plants in alternate rows were sampled for moths. For sampling, each plant was enclosed with a cylindrical mylar cage (55 cm diam. × 90 cm ht.), and was tapped to flush out the moths. The moths were collected using a portable battery operated vacuum cleaner (National BH-611-R) which was modified into a suction sampler (Fig. 1). The number of moths caught was recorded using a hand counter. After completing the collection of moths in each quadrat, the collection apparatus was detached from the vacuum cleaner, and the plastic cup with the screen cage containing the moths was kept in an ice chest for ca 5 to 10 min to immobilise the moths. The screen cage containing the moths was then removed from the cup and the moths were marked with the Solar Pure Blue mark. Marked moths were immediately dried by exposing them to the warm air currents issuing from the vent at the side of the vacuum cleaner. After the moths were adequately dry and no harmful effects were noted, they were released at the center of each quadrat from where they were initially caught. It is assumed that the subsequent

Fig. 1. The modified vacuum cleaner used for capturing and marking adults of *P. xylostella* in the field. (A) screw-on cap, (B) 28-mesh screen cage, (C) 4 mm thick plastic to strengthen joint, (D) vacuum cleaner. All measurements in cm.

activity of the marked moths was normal, although it is not known whether the marking interfered with their responses to external stimuli. Marking and releasing of the moths was done on 10th June 1985, but recaptures were made five times from 13th June 1985 to 21st June 1985. The recaptures were done at equal intervals of two days with the exception of the first occasion. Recaptured moths were released again.

C. *Analytical methods.* The survival rate and population density were estimated using JACKSON's (1939) positive method. At first, the number of recaptures on the i th day (m_i) is converted to an index of recapture y_i as follows:

$$y_i = 10^4 m_i / M_0 n_i \quad (1)$$

Here, M_0 is the number of marked individuals released on the 0th day and surviving on the i th day, and n_i is the number of individuals caught on the i th day. The index y_i is the number of recaptures based on the assumption that 100 marked individuals are released on the 0th day and 100 moths are caught on the i th day. When y_i is plotted against time i , a survivorship curve of marked individuals is obtained when the population is stable. Assuming a constant survival rate over the experimental period, the relationship can be approximated by linear regression:

$$\log y_i = \log y_0 + i \log S \quad (2)$$

where S is the survival rate during a unit time and y_0 is the theoretical value of recapture, when we assume that 100 marked individuals released instantaneously intermingle into the population and that 100 individuals are randomly caught before either disappearance or dilution occurs. The density of moths is then calculated by:

$$N_{j+} = 10^4 / y_0 \quad (3)$$

RESULTS

Laboratory tests

There was no significant difference in longevity between moths treated with the various marks and control (unmarked) moths (Table 1). However, only four dyes, Solar Pure Blue, Acid B.M. Green, Solar R. B. and Acid Scarlet B.A., left marks which were easily recognizable on the legs and body of the moths after wash-off by water droplets; the marks on the wings were not as clear.

In test 2, there was no significant difference between longevity and fertility of moths marked with Solar Pure Blue and Solar R.B. and the unmarked moths (Table 2).

There was no significant difference in the number of mating pairs among the two

Table 2. Longevity and fertility of marked and unmarked *P. xylostella* adults at 25±1°C

Date	Treatment	Longevity (days) (mean±SD)		Fertility (mean±SD)
		Male	Female	
19.4.85	Solar Pure Blue	4.5±2.4	2.9±1.2	32.3±26.1
	Control (unmarked)	4.2±2.0	2.4±0.7	33.6±25.4
24.6.85	Solar R.B.	3.8±1.3	3.3±0.8	71.0±31.5
	Control (unmarked)	4.0±1.9	2.9±0.8	70.8±32.4

No significant difference at $p > 0.05$ was observed between longevity of the two sexes and fertility of females (number of eggs laid per female until death) of marked and unmarked adults using MANN WHITNEY's *U*-test.

Fig. 2. Relationship between the corrected number of recaptures (y_i) and time (i) in days.

marks and control in test 3. The mean number of mating pairs after 1 h was 5.33 ± 0.58 , 5.66 ± 0.58 and 5.00 ± 1.00 for Solar Pure Blue, Solar R.B. and the unmarked moths (control), respectively. We did not observe any abnormalities in mating behaviour among the marked moths.

Field test

Three hundred and two marked adults were released on June 10, 1985, and the number of marked and unmarked adults caught afterwards was as follows:

	June	13th	15th	17th	19th	21st
Marked		24	16	10	1	0
Unmarked		273	393	213	176	143

The relationship between $\log y_i$ and i is shown in Fig. 2. Visual analysis of the graph suggested that the relationship could be approximated to a single line ($\log y_i = 1.06 - 0.17i$, $r^2 = 0.77$). Based on the linear regression, N_{j+} , the number of adults per 400 m² of cabbage on June 10, 1985, was estimated to be 862, and the survival rate of the moth was 0.67 per day.

DISCUSSION

Laboratory studies suggested that it is feasible to use at least two dyes, viz. Solar Pure Blue and Solar R. B., for marking *P. xylostella* adults without noticeable detrimental effects. These marks were easily recognizable on the moths and apparently did not affect their longevity, fertility or mating of the moths in the laboratory. The experiments also suggested that marking on the body and legs was necessary in field studies in which the moths are subjected to rain. In any case, the durability of the marks under field conditions should be investigated in future.

The number of adults at the start of the experiment on June 10, based on JACKSON's 'positive' method was 862. Since we did not census the adult population numbers in the field, it was not possible to assess the accuracy of the estimate. However, using the LINCOLN Index (LINCOLN, 1930) and the number of recaptures on June 13, the estimated number of adults per 400 m² of cabbage on June 10 was 3435. This value could be overestimated because LINCOLN index neglects the effects of mortality and dilution (ITÔ,

1976). However, by multiplying the LINCOLN estimate by daily adult survival rate of 0.67 (see ITÔ, 1976), estimated from JACKSON's method above, the modified LINCOLN estimate is $3,435 \times 0.67^3 = 1,033$. Notwithstanding the low recapture rate, this value was near the JACKSON estimate. Now, if the recaptures on June 15 are considered, the LINCOLN population estimate on June 10 is 7,417 and the modified value is $7,417 \times (0.67^5)$, which is 1,001. The latter value does not differ much from the estimated number of adults using the recaptures of June 13.

The data on actual survival rates of moths in the field are not available, but the estimated survival rate obtained in this study seemed reasonable considering the short life span (<10 days) of the moth under field conditions and the adverse weather conditions, especially rainfall, during the experiment. There is some possibility that the handling (capture and marking) could have had some harmful effect on the moths. In this case, the Jackson estimate should be somewhat overestimated, but there might be no large bias in the estimated survival rate.

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