Using the Mark-and-Release Method in the Estimation of Adult Population of Sweet Potato Weevil, *Cylas formicarius* (FABRICIUS) in a Sweet Potato Field¹

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The object of this study was to develop a procedure for estimating the adult population of sweet potato weevil, *Cylas formicarius* (FABRICIUS), distributed in a relatively small field, by using the mark-release method. Considering the difference of the diurnal activity rhythms between the sexes, the males were captured with a sweep-net at 2200 and the females, by using a sweet potato tuber trap at 1400. The tubers in the trap were inside a covered plastic mesh basket for protection against rats. Weevils could go freely in and out of the basket through the mesh. The capture efficiency differed among the sweet potato cultivars used. Tubers with one third of the epidermis peeled off and left outdoors for a while were more attractive than the fresh ones. The capture efficiency of this trap decreased with the flourishing of plants. The weevils were cooled on a chilled mat (Ice-None®) covered with gauze so as to suppress their activity during marking. They were marked on their forewings with an oily paint-marker. Marking did not have a lethal effect upon the weevils and the loss rate of mark was negligible. PETERSEN and YAMAMURA et al. methods were used to process the mark-release data obtained in the field.

Key words: *Cylas formicarius*, population estimation, mark and release

INTRODUCTION

An estimation of the size of a population of sweet potato weevil, *Cylas formicarius* (FABRICIUS), is the first step in understanding the population processes of this insect in the field. JANSSON and MCSORLEY (1990) provided the sampling programme for a population consisting of larvae, pupae and adults of *C. formicarius elegantulus* (SUMMERS) which were damaging the sweet potato, *Ipomoea batatas* (L.) LAM., plant in the field. PROSHOLD et al. (1986) devised the plastic trap baited with synthesized sex-pheromone (HEATH et al., 1986) to collect male adults of this species. Also JANSSON et al. (1989)

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showed that the seasonal change in the male adult population in a relatively large habitat can be tracked by a census with the aid of this trap. The present study intends to develop a procedure for estimating the adult population of *C. formicarius* distributed in a relatively small-scale sweet potato field, by using the mark-release method.

In developing the procedure, an efficient method of drawing samples from a population should be established first. Behavioural properties of the population are especially important when employing the mark-release technique. The sweet potato weevils have a diurnal activity pattern, rhythmically changing their micro habitats in a sweet potato field in the course of a day (PROSHOLD, 1983; SAKURATANI et al. in press).

In estimating the population with such complicated behavioural patterns, it is essential that both the micro habitat and the timing for obtaining samples be established beforehand. In this study we used the sweep-net method for those of the *C. formicarius* found on the plant crown, and the sweet potato tuber trap method for those on the ground.

The latter method was used on the basis of the observation that the adult weevils swarmed on the tubers that were left in the field. This paper shows that we can successfully estimate the population size of this pest in a relatively small field by combining the mark-release technique with these two methods of collecting samples.

MATERIALS AND METHODS

1. *Methods of capturing weevils*

Properties of the sweet potato tuber trap

The surveys were carried out in August 1989 in three fields (A: 3.7 a, B: 13.6 a, C: 1.5 a) of sweet potato (cultivar: Koganesengan) at the Ohshima Branch, Kagoshima Agricultural Experiment Station.

Types of tuber trap. First, to compare the trap efficiency between the two different cultivars, Koganesengan and Kokei 14, used as bait, 15 pairs of traps consisting of three tubers of each cultivar were arranged in the field at 8 m intervals along two lines, 40 cm apart. The numbers of trapped weevils were counted at 1400 every day for 14 days. Next, to establish the relationship of the number of tubers used as bait to the capture efficiency, traps consisting of 1, 3, 6 or 10 tubers were arranged in a latine square at 2-m intervals in field A. The number of the weevils collected on each of these traps was counted at 0900 every day from August 21 to 25. Next, to determine the effect upon the capture efficiency of the time elapsed after the removal of the tubers from storage and of the degree of damage to the tubers, the following four types of tuber traps (consisting of five tubers each) were arranged in a latine square at intervals of 6 m in field B. The number of collected weevils was counted from August 29 to September 2. A third of the epidermis of half of the tubers used was peeled off after their removal from storage (15°C). Each of the halves was put out in the field either just after storage or after being kept outdoors for one week after being taken out of storage. In this case, trapped weevils were counted at 0600, 1000, 1400, 1800 and 2200 every day and were then placed back on the ground with the trap, because of the need to study their diurnal activity rhythms as described below.

Effect of plants upon weevil capture. Two mesh cages (2×2×2 m), one with five potted sweet potato plants (about 1-m length, with about 50 leaves) and the other without any plants, were placed on the ground. Sixty females were released into each

cage. One other tuber trap contained three tubers that had been kept outdoors for two weeks. Weevils collected by the trap were counted at 1400 every day for ten days and released again into the cages after counting.

Diurnal change of weevil capture

The method of studying the diurnal change of the number of weevils captured in the tuber trap was already described above. Next, to determine the number of weevils captured by sweep nets, the weevils found on plant crowns were collected with sweep nets (36-cm net opening, with a 120-cm handle) in field C at 0600, 1000, 1400, 1800 and 2200 every day from August 29 to September 3. Ten approximately equally-spaced sampling points were selected, and at each point ten sweeps of the net were made. The netted weevils were released at each point after counting.

2. *Marking*

Weevils which had emerged as adults 15 days earlier in the insectarium (27°C) were cooled on a chilled mat (Ice-None®, Hakugen Co.) to reduce their activity while marking. The marks were applied to their forewings using an oily paint-marker (Sakura Paint-Marker®, Sakura Color Products Corp.).

Effect of marking upon weevil survival

Fifty each of the marked and unmarked weevils were reared with a chip of sweet potato tuber in a plastic container (6-cm ht. × 9-cm dia.) at 27°C for 53 days.

Loss of mark

Two hundred marked males were released into a mesh cage (2 × 2 × 2 m) containing planted sweet potato plants on November 22, 1991. There they were isolated from the outside weevil population by the mesh. Using the pheromone trap (SETOKUCHI et al., 1991) the males were collected at 10-day intervals and released after checking of marks.

3. *Population estimation*

The mark-release estimation procedure was carried out on two sweet potato fields (about 5 a each, cultivar: Koganeshengan planted on April 26, 1990) in Naze, Amami-Oshima Is.

Capturing weevils

The tuber trap was used to collect females (see text). The tubers, with a third of their epidermis peeled off, were kept outdoors for one week after being removed from storage. Three tubers per trap were placed into a plastic mesh basket (25-cm ht. × 27-cm dia.) with a lid so as to protect them from rat damage. Weevils can go freely in and out of the basket through the mesh. Twenty traps each were positioned in two fields, as equally spaced as possible. Next, the sweep-net method was applied to catch the males. Eleven and 15 sampling points in the surveys I and II, respectively, were selected, at a distance as equal as possible, and at each point a ten-stroke sweep-net sample was taken.

Marking

Ten-day-old, already mated, weevils reared in the insectarium (27°C, 60–80% RH) had their forewings marked with the paint marker. Each 50 weevils had been maintained in a plastic container (6-cm ht. × 9-cm dia.) with a chip of sweet potato prior to their being released in the field. Weevils released later were kept in the container for about one week in each of the surveys.

Survey method

In survey I, on August 2 and on August 6, respectively, 1000 pairs and 500 pairs of

marked weevils were released in the field, as uniformly as possible, and recaptured by the tuber trap on August 7 and by the sweep-net on August 8. In survey II, 1000 pairs of marked weevils were released on September 21 and 500 pairs on September 24 and were then recaptured by the tuber trap on September 25 and by the sweep-net on September 28. In these surveys different marks were made for different release dates. By considering the diurnal activity of this weevil (see text), we counted the number of trapped weevils at 1400 for the tuber trap and checked the sweep-net samples at 2200. After being counted, the weevils captured by the tuber trap were again placed on the ground with the trap, and those caught by the sweep-net were again released at the sampling points where they had been taken.

RESULTS AND DISCUSSION

1. Methods of capturing weevils

Properties of tuber trap

Types of trap. The cultivar Koganesengan was superior in capture efficiency to Kokei 14 (Table 1). Also, the capture efficiency increased with increasing number of tubers used per trap (Table 2). It is noteworthy that few males were captured in this case. According to the observation, tubers that were kept outdoors for a while appeared to become more attractive to the weevils. The field test proved that tubers that were kept outdoors after a portion of their epidermis had been removed were the most attractive (Table 3).

Effect of plants upon weevil capture. Only 20 weevils were caught over a 10-day period in a cage with potted sweet potato plants as compared with 93 for a cage without the plants ($\chi^2=46.9$, $p<0.01$). The capture efficiency of the tuber trap would decrease markedly as a result of the plant effect. Thus, because the plant effect increases with

Table 1. Efficiency of capturing weevils in two different cultivars used as a trap, Koganesengan and Kokei 14 (mean $\pm$ SD)

Cultivars	n	No. captured/trap/day		
		Females	Males	Total
Koganesengan	210	4.4 $\pm$ 8.4	2.3 $\pm$ 3.8	6.7 $\pm$ 11.9
Kokei 14	210	1.3 $\pm$ 1.8	0.7 $\pm$ 1.2	2.0 $\pm$ 2.2

Significantly different between cultivars in females, males and total at the 5% level by STUDENT's *t*-test.

Table 2. The relation between the number of tubers per trap and the mean number of female weevils trapped

No. of tubers per trap	n	Mean no. of trapped weevils (per trap per day)
1	20	1.6 ^a
3	20	2.0 ^a
6	20	5.8 ^b
10	20	9.0 ^b

Means followed by a different letter are significantly different at the 5% level by TUKEY's test.

Table 3. The relation between types of tuber trap and total number of females collected by four traps and its ANOVA

Types of trap		No. of collected females				
	Epidermis	29 Aug. (B ₁)	30 Aug. (B ₂)	31 Aug. (B ₃)	1 Sept. (B ₄)	2 Sept. (B ₅)
Just after release (A ₁)	not peeled (C ₁)	0	5	12	11	21
	peeled (C ₂)	1	15	16	25	20
One week after release (A ₂)	not peeled (C ₁)	3	7	9	20	18
	peeled (C ₂)	9	14	24	42	38

Source of variation	SS	df	MS	F
Between A	168.2	1	168.2	8.76 ^a
Between B	1341.0	4	335.3	17.46 ^b
Between C	480.2	1	480.2	25.01 ^b
A × B	93.8	4	23.5	1.22
A × C	88.2	1	88.2	4.59
B × C	108.8	4	27.2	1.42
Error	76.8	4	19.2	

^{a, b} Significantly different at the 5% and 1% level, respectively.

Fig. 1. Diurnal changes in numbers of female weevils of *C. formicarius* captured by tuber traps (top), and female (solid) and male (broken) weevils captured by sweep-net (bottom) in the sweet potato field.

the flourishing of plants, direct count using this trap is not recommended as a measure of the seasonal changes of the population density of this weevil. However, this does not necessarily mean that it is wrong to employ this trap as a means of collecting the weevils in a mark-release estimating process, because for this estimate one needs only

the estimated ratio of the marked and the unmarked insects, without considering the estimation efficiency.

Diurnal changes of weevil capture

We captured 292 weevils with a total of only three males when using the four types of the tuber trap for a period of five days. The number of trapped females changed rhythmically over the course of one day. The females were fewer at night, their number peaking at around 1400 h (Fig. 1, top half). Fewer females were captured by the sweep-net method (Fig. 1, bottom half). The number of males captured by the sweep-net changed rhythmically within one day, being lower during daytime and reaching a maximum around 2200 h (the significant serial correlation coefficient was obtained at a time lag of 24 h in both trap counts) as HOWARD (1982) reported in *C. formicarius elegantulus*. A similar change in the number of captured males was found in the case of the sex-pheromone baited trap (PROSHOLD et al., 1986; YASUDA et al., 1992). Such diurnal rhythms in the numbers of captured weevils reflect the diurnal rhythms in the activity of this weevil (SAKURATANI et al., in press), which closely resembles that of the *C. formicarius elegantulus* (PROSHOLD, 1983). SAKURATANI et al. (in press) released marked weevils into cages containing sweet potato plants and observed their behaviour. At night, most females got into the soil through a crack for oviposition or went up to the plant crown for copulation. At daytime, many females, especially those that had already mated, burrowed into the soil or remained on the ground under the crown. However, some of the unmated females were found to remain on the crown at daytime. On the other hand, most males climbed up onto the crown for copulation at night, and moved down onto the ground in the daytime.

Figure 1 predicts that the females should be captured by the tuber trap at 1400 and the males by the sweep-net at 2200, since the estimation efficiency of the population density increases with the number of captured individuals. The exact proportion of marked weevils can be estimated when they are thoroughly intermixed with the wild ones in the population and are captured with a probability equal to that of the wild ones. According to observations by SAKURATANI et al. (unpublished), marked males seem to have diurnal rhythms of activity similar to those of the wild males, and so will be captured with a similar probability on the plant crown at night together with the wild ones. However, we released the already mated females as we did the marked ones. During daytime, mated females would have descended to the ground, while a portion of the unmated wild females would have remained on the plant crown as described above, and a proportion of marked females captured by the tuber trap will be slightly overestimated in this situation. This bias will be especially large in a growing population which contains many unmated, newly emerged females.

2. Marking

Effect of marking upon weevil survival

Total mortality of marked weevils was not any different from that of the unmarked ones (Table 4). The effect of marking upon weevil survival was negligible.

Loss of mark

We recaptured 448 marked weevils (weevil-days) for 50 days. On 18 (4%) of them the mark on their forewing could not be directly recognized with a naked eye. But we could recognize a trace of the mark through a binocular.

Table 4. Effect of marking upon survival of weevils

	No. weevils tested	Accumulated no. of dead weevils after marking	
		By 30th day	By 53rd day
Marked	50	9	30
Unmarked	50	4	27

Not significantly different for both dates at the 5% level by G-test.

Table 5. Mark-release data of sweet potato weevils (top: female, bottom: male)

Dates	Survey I			Dates	Survey II		
	Unmarked	White	Blue		Unmarked	White	Blue
8/2		<i>1000</i>		9/21		<i>1000</i>	
6			<i>500</i>	24			<i>500</i>
8	22	34	2	26	57	32	6
11	19	36	3	10/1	147	54	6
14	17	17	2	6	77	40	13
17	26	12	3	11	68	16	8
20	22	6	4	16	56	16	5
				20	27	10	3

Dates	Survey I			Dates	Survey II		
	Unmarked	Pink	Yellow		Unmarked	Pink	Yellow
8/2		<i>1000</i>		9/21		<i>1000</i>	
6			<i>500</i>	24			<i>500</i>
8	48	17	14	29	37	12	4
11	31	5	15	10/4	74	10	6
14	45	15	16	9	64	10	4
17	23	3	7	13	88	6	6

Italics show the number of marked and released weevils.

Table 6. Estimation of population density of sweet potato weevils

		Survey I	Survey II
Female	Np(W)	647.1	1781.3
	Np(B)	5500.0	4750.0
	Ny(W)	792.3	1165.5
	Ny(B)	9552.6	1841.8
Male	Np(P)	2823.5	3083.3
	Np(Y)	1714.3	4625.0
	Ny(P)	1285.9	1284.7
	Ny(Y)	2844.0	3468.8

Np: Estimates from the first sampling data by PETERSEN method. Ny: Estimates by YAMAMURA et al. method.

Letters in parentheses: Colours of marks. W: White, B: Blue, P: Pink, Y: Yellow.

3. Population estimation

The recapture data are shown in Table 5. PETERSEN's method (SEBER, 1982) was applied to these data (Table 6). Also, YAMAMURA et al. method (1992) was applied for a single mark and two recaptures. In using this method we adopted $\phi = u_1 m_2 / (m_1 u_2)$ and $\hat{U} = \hat{\phi} M_0 u_1 / m_1$ because we had again released all the captured weevils in the field, contrary to the premise of this method. The estimates by PETERSEN's method were obtained from the first set of recapture data in each survey. When estimating the density of the same population by the two different marks, the discrepancy between the two estimates was about two-fold in many cases. The large discrepancy of estimates by the white-marked and the blue-marked females in survey I is due to the lower recapture rate of the blue marks. This lower recapture rate may be the result of their low activity following a longer confinement in a small and crowded container which would have left them in a weakened state. This point should be improved. In this study the recaptured females were again returned to the ground with the tubers after counting. The females would tend to gradually concentrate around the tubers after being released, indicated by the subsequent daily increase of captures of females by tuber traps (Fig. 1). In this case, the recapture rate of the marked weevils that had been released later will be lower than that of the ones released earlier since we recaptured both marks on the same days regardless of the date of their release. This concentration tendency will lead to a slight bias in the mark-release estimation. Under such conditions it would be better to disperse the captured females randomly in the studied field, or to remove them.

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