Suppression of the Melon Fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae), on Kudaka Is. with Sterile Insect Releases

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Prior to the beginning of a project to eradicate the melon fly, *Dacus cucurbitae* COQUILLET, from Kume Is., a pilot release experiment was made on Kudaka Is. from November 14, 1974. A tentative estimate of the total number of wild melon flies in October was ca. 10,000. The ratio of sterile to wild males on Kudaka Is. during the study period reached a maximum of 8.23, when we released ten times as many sterile flies as the estimated number of wild flies in each of 9 weekly releases. Percent hatch of eggs collected from loofahs on Kudaka Is. decreased from 55.6 on December 11 to 7.0 on January 14. On the other hand, the percent egg hatch in the control area on the Island of Okinawa was 45.8 to 86.7%. The pupae released from release buckets were seriously attacked by mammalian predators, such as rats and mice. Estimated mortality of pupae by predators increased from 24.7% on the first release to 84.6% in the 4th release. In spite of high pupal mortality, the main reason for the good ratio of sterile to wild males was considered to be the seasonal decline in the wild population. The results obtained suggested that the ability of sterile males to compete with wild males was not so remarkably decreased under fluctuating field conditions.

INTRODUCTION

Prior to the beginning of a project to eradicate the melon fly, *Dacus cucurbitae* COQUILLET, from Kume Is., Okinawa Prefecture, a pilot release experiment was made on Kudaka Is., a small islet 122 ha in area, situated 6 km east from Chinen Peninsula, which is the southern part of the Island of Okinawa. This flat islet is 600 m in width at the widest part and 3,400 m long (Fig. 1). About 500 people live in a small village in the southern part of the islet where Chinese cucumbers or loofahs (the major host of the melon fly on this island) were cultivated. This paper reports on experiments to estimate the population density of melon flies on Kudaka Is. and to determine longevity, mortality due to predation, and apparent sexual competitiveness of released sterile flies.

MATERIALS AND METHODS

Population Estimate

Flies used in these experiments were reared at a constant temperature of 27°C in a mass rearing facility of the Yaeyama Branch of Okinawa Prefectural Agricultural Experiment Station on Ishigaki Is. and then transported by air to Naha on Okinawa

Fig. 1. Points of release of sterile flies (solid circle) and traps for monitoring the adult population (hollow circle). Striped area indicates village and area in square indicates 'station' to estimate the population density with the mark-recapture method.

Is. Newly emerged flies were fed protein hydrolyzate, yeast-extract, sugar and water. Male flies less than 1 week of age were marked with lacquer using a double anesthetization technique (IWAHASHI, 1972). These marked males were released on Kudaka Is. near the center of a rectangular "station", 6.25 ha in area, in which the population estimate was made (Fig. 2), and recaptured with 25 "lantern" type plastic traps with 4 holes (Fig. 3). These traps which were baited with 5 ml of a solution 97% cue-lure and 3% naled were placed at intervals of ca. 50 m (Fig. 2). The number of individuals on the station was estimated by the Petersen-Lincoln method (see Appendix).

On the other hand, from September 18 to 28, 10 and 5 traps were located inside and outside the village, respectively. The number of flies caught in these traps were used for a tentative estimate of the total number of melon flies in Kudaka Is.

Sterile-insect Release

When the pupae sent from the Yaeyama Branch of the Okinawa Prefectural Experiment Station reached 7 days of age under a constant temperature of 27°C, they were irradiated with 7 Krad. Irradiations were made with a 5,000 Ci cobalt-60 source at the center of a flat desk. Pupae held in the containers, 30 × 15 × 3 cm³, of which either sides were saran-net, were set on turn tables (10 turns per min) and irradiated for a given time. TERUYA et al. (1975) showed that this dose resulted in complete sterility of females and almost complete sterility of males without any significant reduction in longevity under laboratory conditions. The pupae were then marked with fluorescent powder at a rate of 3 g/l of pupae. Different colors of fluorescent powder, viz, Tinopal, Satan Yellow, Blaze Orange and Rhodamine Red were used to mark pupae in order to determine the relative longevity of sterile flies released on different days. Adult eclosion took place about 3 days after irradiation. Releases of pupae were repeated 9 times at weekly intervals from nine points (Fig. 1), using buckets (Fig. 4). Approximately 11,000 pupae were placed in each bucket. The pupae were

Fig. 2. Map of stations to estimate the population density with the mark-recapture method. R indicates release point, solid circles indicate traps to estimate density and open circles indicate traps to monitor population trend.

Fig. 3. A trap used to monitor the adult population.

Fig. 4. A bucket used to release sterile melon fly pupae.

left in the release buckets to the day of the next release, when samples were taken to determine percent emergence. To monitor the adult population 15 plastic traps baited with cue-lure (97%) and naled (3%) were distributed throughout the

islet (Fig. 1 and 2) and examined weekly. Heads of all the flies caught in the traps were crushed on filter paper and checked under an ultra-violet lamp 3650 Å/100 V) to determine the ratio of marked (sterile) to unmarked (wild) flies.

Fruits of host plants (mainly loofahs) on the islet were collected weekly and dissected to find eggs. These eggs were kept for 2 or 3 days on a sheet of moistened filter paper in a petri dish at a constant temperature of 27°C and examined for hatch under a binocular microscope. The area of the Okinawa Prefectural Agricultural Experiment Station on Okinawa Is. was used as an untreated check.

RESULTS

Population Estimate

The methods of estimation of the number of wild males in this study is shown in detail in the Appendix. The number of wild males in the station of 6.25 ha, which was situated in the village where the density of the melon fly was relatively high, was estimated to be ca. 500 to 1,000 (80 to 160/ha) on the middle of October. The overall density in the village was considered to be about the same as that in the station.

On the other hand, from September 18 to 28 the number of flies caught in traps located inside and outside the village were 1,110 and 220 per 1,000-trap-days, respectively (Table 1). From these values the density of the melon fly in areas outside the village was estimated to be about one-fifth that within the village, i.e., 16 to 32/ha. The areas inside and outside the village are 10 and 112 ha, respectively. Then, if we use the larger values of 160/ha within the village and 32/ha outside the village, the number of wild males (N) in Kudaka Is. is:

$$\begin{aligned} N &= (160 \times 10) + (32 \times 112). \\ &= 5,184 \end{aligned}$$

Thus, a tentative estimate of the total number of melon flies in Kudaka Is. in October was ca. 10,000.

Sterile-insect Releases

Sterile flies were released weekly at a rate of 100,000 per week from November 14, 1974 to January 14, 1975. The number of sterile insects released was therefore about 10 times the number of wild (fertile) ones. The results of the releases are shown in Table 2. The first release made on November 14 resulted in a ratio of sterile to fertile males of 1.41. Thereafter the ratio increased slowly with subsequent releases. The ratio was 3.17 on December 21. On January 14, 2 months after the first release, the ratio reached the highest value of 8.23. The final release of sterile insects was made on that day. After the last release, high values of the sterile : wild ratio were maintained for one month, that is, 6.07 on January 29, 7.64 on February 5, and 6.78 on February 13. Thereafter the ratio decreased rapidly to 1.15 on March 6, 1975.

Data on percent hatch of eggs collected from loofahs on Kudaka Is. and in the control area on the Island of Okinawa are also shown in Table 2. On December 11, 50 of 90 eggs hatched (55.6%). On January 1, the percent egg hatch decreased to 24.5 and on January 14, a further decrease to 7.0% was recorded. This low value of egg hatch continued during the subsequent two surveys, that is, 11.1% on January 29, and 9.2% on February 5. Egg hatch started to increase thereafter and on March 6, 87% hatch was recorded. On the other hand, the percent egg hatch in the un-

Table 1. NUMBER OF FLIES CAUGHT IN TRAPS INSIDE AND OUTSIDE THE VILLAGE, KUDAKA Is., SEPT. 18-28, 1974

	No. flies caught in traps	
	Inside the village	Outside the village
	6	4
	11	2
	4	1
	6	0
	1	4
	39	
	15	
	7	
	0	
	22	
Total	111	11
No. flies/1,000 trap/day	1,110	220

Table 2. MELON FLY CATCHES, RATIOS OF STERILE TO WILD MALES AND PERCENT EGG HATCH AS A RESULT OF STERILE MALE RELEASES ON KUDAKA Is.

	No. males released ^a	No. males trapped		Ratio of S:F-males	Treated area (Kudaka Is.)			Untreated area (Okinawa Is.)		
		Sterile	Fertile (Wild)		No. eggs examined	No. eggs hatched	% eggs hatched	No. eggs examined	No. eggs hatched	% eggs hatched
Nov. 14 1974	50,000									
Nov. 28	50,000 ^b	370	262	1.41						
Dec. 5	50,000	239	129	1.85						
Dec. 11	50,000	931	177	5.25	95	50	55.6			
Dec. 21	50,000	374	118	3.17						
Dec. 27	50,000	273	44	6.20	205	108	52.7	10	8	80.0
Jan. 1 1975	50,000	300	79	3.80	40	12	24.5	27	20	74.0
Jan. 8	50,000	197	36	5.47	—	—	—	—	—	—
Jan. 14	50,000	107	13	8.23	113	8	7.0	52	28	53.8
Jan. 21		—	—	—	—	—	—	—	—	—
Jan. 29		265	38	6.97	18	2	11.1	15	13	86.7
Feb. 5		275	36	7.64	98	9	9.2	50	28	56.4
Feb. 13		61	9	6.78	152	60	39.5	24	11	45.8
Feb. 20		21	12	1.75	—	—	—	—	—	—
Feb. 27		14	7	2.00	274	0	0.00	44	38	86.4
Mar. 6		15	13	1.15	23	20	87.0	25	20	80.0

^a One hundred thousand pupae including both sexes were released weekly.^b Emerging flies were released.

treated area was always higher than that on Kudaka Is. These values ranged from 50 to 80% and the lowest egg hatch was 45.8% on February 13.

These results suggest that if releases of sterile insects were continued for several

Table 3. MORTALITY OF PUPAE DUE TO PREDATION BEFORE ADULT ECLOSION

	Nov. 14	Nov. 28	Dec. 5	Dec. 11
No. pupae released	50,000	50,000	50,000	50,000
Estimated no. eaten before adult eclosion	12,348	0	24,110	42,341
No. valid pupae released	37,651	50,000	25,290	7,686
Percent emergence	55.2	88.5	84.6	63.6
No. valid adults released	20,784	44,250	21,903	4,888
No. flies recaptured	557	1,186	587	131
Estimated percent mortality by predation	24.7	0.0	48.2	84.6

more months, the population density of the melon fly on Kudaka Is. might have approached a level near extinction.

During the course of this study pupae placed in the release buckets were often eaten by mammalian predators, such as rats and mice. Table 3 shows a procedure used to estimate the mortality of pupae due to predation. On November 28, a delayed release was made so that flies were emerging when the buckets were set. Under these conditions mortality of pupae by predators should be negligible, thus we can consider that the "valid" (effective) number of pupae was ca. 50,000 on this occasion. The total number of males recaptured during the subsequent four weeks after the release made on November 28 was 1,186. Examination of pupae remaining in the bucket showed that the adult emergence was 88.5%. Thus the valid number of released males was estimated to be:

$$50,000 \times 0.885 = 44,250$$

The rate of recapture was

$$1,186 \times (1/44,250) = 0.0268$$

We used this value as an estimate of the rate of recapture of sterile males when there was no predation during the pupal period. Using this value, the mortality of pupae by predators was estimated as follows:

For flies released on November 14, the number of males recaptured was 577. If the rate of recapture was the same as that of flies released on November 28, the valid number of males released was

$$577 \times (1/0.0268) = 20,784$$

The adult emergence was found to be 55.2%. Then the valid number of pupae should be:

$$20,784 \times (1/0.552) = 37,652$$

The number of pupae eaten by predators was thus estimated to be:

$$50,000 - 37,652 = 12,348.$$

The mortality of pupae by predators was therefore:

$$12,348 \times (100/50,000) = 24.7\%$$

By following the above steps, the mortality due to predation was calculated to be 48.7% on December 5, and 84.6% on December 11. The valid number of males released was 21,903 on December 5 and only 4,888 on December 11.

The temporal sequence of recaptures of irradiated flies, which indicates the relative longevity of flies, is shown in Table 4. Flies dyed with Satan Yellow and released on November 28 could not be differentiated after the fourth week since all the flies released after December 11 were also colored with Satan Yellow. The first week's survey

Table 4. RECAPTURES OF STERILE FLIES RELEASED ON DIFFERENT DATES

Date	Nov. 14	Nov. 28	Dec. 5	Dec. 11
Dyes	Tinopal	Satan Yellow	Blaze Orange	Rhodamine Red
No. males released	50,000	50,000	50,000	50,000
Days after release (week)				
1	—	76	15	9
2	370	891	207	17
3	162	153	190	80
4	25	66	175	25
5	5	—	15	5
6	0	—	13	6
7	0	—	34	6
8	3	—	29	1
9	0	—	8	1
10	0	—	0	1
11	3	—	3	2
Total	568	1,186	689	153

of flies released on November 14 was not conducted because of rough sea conditions. In the second and third weeks 370 and 162 flies, respectively, were recaptured. After the fifth week only a few flies were recaptured. Flies released on December 5 and 11 were recaptured almost continuously for 11 weeks. There was no recapture of flies marked with any dye other than Satan Yellow since the 11th week.

DISCUSSION

The ratio of sterile to wild males on Kudaka Is. during the study period reached a maximum of 8.23, when we released ten times as many sterile flies as estimated number of wild flies in 9 weekly release. The percent hatch of eggs obtained from host fruits also declined as the ratio of sterile males increased (Table 2). These results suggest that the population estimate, though it was very tentative, was reasonably accurate, and that the sterile insect release method for melon fly eradication should be very effective if the program is conducted under ideal conditions. However, it is unlikely that the melon fly on Kudaka Is. would have been eradicated completely even if sterile insects were continuously released after January 14, because of the possibility of emigration from the Island of Okinawa, which is only 6 km from Kudaka Is.

It was noted that once a release bucket was attacked by predators the pupae released at that site were always seriously attacked, apparently due to learned behavior of predators. Thus it has been suggested that the buckets should be relocated some distance (e.g. 100 m) at each release. The mortality of pupae by predators increased from 24.7% in the first release to 84.6% in the 4th release (Table 3). Although data on the rate of predation on pupae released after December 21 were not obtained this high mortality rate probably did not decrease, and the number of valid flies in each of the subsequent 4 releases was considered to be approximately one-tenth of the number of released pupae. If so, what were the reasons for the increase in ratios of sterile to wild males and the decrease in percentage of egg hatch during December and January?

The number of wild flies caught in traps decreased to one-twentieth from November 28 to March 6 (Table 2). This suggests that the population density of the melon fly was decreased considerably during the experimental period. Thus the decrease in the valid number of flies released was considered to be compensated for by the seasonal decrease in the number of wild flies.

The rate of egg hatch on Kudaka Is. decreased to a level of about 10 percent during a period, when that in the control area was about 65%. This fact suggests that the ability of sterile males to compete with wild males is not so adversely affected under the field conditions, though laboratory experiments (TERUYA, in preparation) showed a slight decrease of sexual competitiveness of flies irradiate with 7 Krad during the late pupal stage.

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LITERATURE CITED

- IWAHASHI, O. (1972) Movement of the oriental fruit fly adults among islets of the Ogasawara Islands. *Environ. Entomol.*, **1**: 176-179.
- TERUYA, T., H. ZUKEYAMA and Y. ITÔ (1975) Sterilization of the melon fly, *Dacus cucurbitae* COQUILLETT with gamma radiation: Effects on rate of emergence, longevity and fertility. *Appl. Ent. Zool.* **10**: 289-301.

APPENDIX

Estimation of the Number of Wild Males with a Mark-Recapture Method

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Laboratory reared male flies marked with lacquer on the dorsal part of thorax were released into the field and recaptured by 25 plastic traps baited with cue-lure and naled. The points of release and recapture covered 4-ha area at the southern (populated) part of Kudaka Is. Release was repeated three times using different colours of marks. The results are shown in Table 5.

PETERSEN-LINCOLN estimates of the number of wild males were obtained by the following equation:

$$N_P = \frac{U_1 M_0}{m_1} \quad (1)$$

where M_0 means the number of males released, U_1 means that of unmarked individuals

Table 5. RESULTS OF MARK-RECAPTURE EXPERIMENTS

Date	Unmarked	Green	Blue	Red	Total
Oct. 18	—	1082			—
Oct. 21	247	180	820		427
Oct. 29	222	34	170	418	426
Nov. 5	70	0	6	43	119

Italic figures indicate the number of individuals released.

(male only) caught on day 1 (note that our estimation was based on the number of *unmarked* individuals U , not on the total number of marked and unmarked individuals C , because we added the laboratory-reared flies into natural population), and m_1 means that of marked individuals recaptured on day 1. The N_P s obtained were 1485 for experiment 'green', 1070 for experiment 'blue' and 680 for experiment 'red.' But it must be noted that one of the prerequisites of PETERSEN-LINCOLN method was the absence of mortality, emigration and dilution (emergence and immigration), and this prerequisite was not held in our experiments.

Let's consider the effect of these factors. If P means the survival rate, B the dilution rate and s the rate of capture, the results of PETERSEN-LINCOLN estimation can be tabulated as in Table 2. The survival rate and the rate of capture of the marked individuals are of course assumed to be the same to those of unmarked individuals.

In Table 6, as P and B are positive values ($0 \leq P \leq 1$; $0 \leq B$), it is clear that the PETERSEN-LINCOLN method never gives a negative bias. The estimate of the number of individuals is either correct or overestimated. In case (3) which is considered to be the commonest case in insects, intensity of overestimation is determined by the ratio B/P and when the population density is stable during the period from time 0 to time 1 (case (3')), the intensity of overestimation is proportional to the reciprocal of the survival rate.

Thus, for the aim of determination of the number of sterile males to be released, the PETERSEN-LINCOLN method can give a 'conservative' value. But if we like to know more reliable value of the number of wild males, we have to know the survival rate.

A modified JACKSON's positive method (Itô, 1973) was thus applied to estimate the survival rate and more reliable number of wild males. The equations are:

$$y_i' = \frac{10^4 m_i}{M_0' C_i} \quad (2)$$

$$M_0' = M_0 - \sum_{j=1}^{i-1} m_j \quad (3)$$

$$\log y_0' = \log y_i' + i \log S \quad (4)$$

$$N_I' = 10^4 / y_0' \quad (5)$$

where i means the number of days from the release, C_i means total number of marked and unmarked flies caught on the i th day and S the survival rate (in decimal) during a unit time. As we like to estimate the number of wild flies, we use the following equation:

$$N_I = N_I' - M_0 \quad (6)$$

These equations are based on the assumption that the $\log y_i$ decreases linearly with the increase of time i . We can not determine whether this was true or not be-

Table 6. EFFECTS OF MORTALITY AND/OR EMIGRATION AND DILUTION (EMERGENCE AND/OR IMMIGRATION) ON THE PETERSEN-LINCOLN ESTIMATES

	N_1	M_1	U_1	m_1	N_p
(1) Mortality/emigration occurs but no dilution	PN_0	PM_0	sPN_0	sPM_0	$\frac{sPN_0M_0}{sPM_0} = N_0$
(2) Dilution occurs but no mortality/emigration	$N_0(1+B)$	M_0	$sN_0(1+B)$	sM_0	$\frac{sN_0(1+B)M_0}{sM_0} = N_0(1+B)$
(3) Both the mortality/emigration and dilution occur	$N_0(P+B)$	PM_0	$sN_0(P+B)$	sPM_0	$\frac{sN_0(P+B)M_0}{sPM_0} = N_0(1+B/P)$
(4) Ditto to (3), but in reverse order	$PN_0(1+B)$	PM_0	$sPN_0(1+B)$	sPM_0	$\frac{sPN_0(1+B)M_0}{sPM_0} = N_0(1+B)$
(3') $P+B=1$ in case (3)	N_0	PM_0	sN_0	sPM_0	$\frac{sN_0M_0}{sPM_0} = N_0/P$

cause we recaptured marked flies only twice, but our previous experiments (Itô et al. 1974) showed that this assumption is generally satisfied in the melon fly. Thus we calculated two values of y_i 's (y_1 and y_2) for each of 'green' and 'blue' flies and estimated y'_0 by linear extrapolation of $\log y_i$ lines (Fig. 5). As has been written by Itô (1973), the slope of the line is approximately equal to the instantaneous mortality rate. The calculated survival rate was 0.88 per day for 'green' flies. Using y'_0 indicated in Fig. 5, we calculated N_I for 'green';

$$N_I = \frac{10^4}{5.62} - 1082 = 697$$

Calculation for 'blue' gave the survival rate being 0.80 which resulted in a negative value of N_I . This may be due to the sampling error when we used only two values of y_i ; that is, m_8 (recapture on October 29) was disproportionally large and m_{15} (recapture on November 5) was small. Although the value for 'green' is subject of sampling error, too, we adopted the value for 'green' here, because the similar survival rate

Fig. 5. For explanation, see text.

was obtained in our previous experiments (mean survival rate=0.83 per day—Ito et al. 1974).

It has been shown that N_I still has negative bias, but the intensity of bias is smaller than that of N_P (Ito, 1973). HAMADA (unpublished) developed another method to estimate the density of unmarked individuals, where the negative bias never exceed 5 percent in usual conditions.

$$z_i' = \frac{10^4 m_i}{M_0' U_i} \quad (7)$$

$$\log z_i' = \log z_0' + i \log S \quad (8)$$

$$N_H' = 10^4 / z_0' \quad (9)$$

Using these equations the following results were obtained.

$$10^4 / 11.5 = 870 \text{ for 'green'}$$

and

$$10^4 / 87.1 = 115 \text{ for 'blue'}$$

Survival rate for 'green' was 0.84/day and that for 'blue' was 0.76/day.

Assuming that there was no significant change in the natural density of males from October 18 to November 5, an estimate of true density can be obtained by multiplying N_P by the survival rate p^t . If we use 0.85 as the mean survival rate per day, we can calculate:

$$N(\text{for 'green'}) = 0.85^3 N_P = 912$$

$$N(\text{for 'blue'}) = 0.85^8 N_P = 292$$

$$N(\text{for 'red'}) = 0.85^7 N_P = 218$$

It is more natural for our situation (see the 2nd column of Table 5) to suppose that the dilution rate was smaller than the survival rate. If we suppose that B/P (in case 3 of Table 6) was 0.5, our estimates are:

$$N(\text{for 'blue'}) = N_P - 0.5 N_P = 535$$

$$N(\text{for 'red'}) = 340$$

If the decrease in the density of wild males actually took place, high trapping mortality may be responsible to a part of the decrease.

To sum up, we can present a rough estimate of the number of wild males; that is 500 to 1000 (80~169/ha) in the middle of October. The figure for late-October might be less than this, say 250 to 500 (40~80/ha). We estimated the density of males in peak seasons in Kume Is. and Ishigaki Is. being 200~700/ha and 300~600/ha, respectively (Ito et al. 1974). General impression showed that the density of the melon fly in Kudaka Is. in October, 1974 was somewhat lower than that in the above-mentioned islands. Thus the values we obtained are not unreasonable. This is the base of our determination of the number of sterile males released into Kudaka Island (see p. 103)

LITERATURE CITED

- Ito, Y. (1973) A method to estimate a minimum population density with a single recapture census. Res. Popul. Ecol., **14**: 159-168.
 Ito, Y., M. MURAI, T. TERUYA, R. HAMADA and A. SUGIMOTO (1974) An estimation of population density of *Dacus cucurbitae* with mark-recapture methods. Res. Popul. Ecol., **15**: 213-222.