compatible with this expectation: the number of oocytes began to decrease in mid June (Table 1). Older females may be more prone to be attracted to flowers or MPA than younger ones.

This study shows that the MPA traps collected more females than males throughout the season and that many of the females had eggs. However, the catch of the females was relatively small in May when they had more eggs or had higher reproductive values than they would later in the season. Then, the chemical seems to attract mainly females that have already laid various quantities of eggs. IGARASHI and IGARASHI (1988) also found that female adults collected on flowers had lower longevities and laid fewer eggs than newly emerged females under the same rearing conditions. Thus, the female may visit flowers (or get attracted by their odors) after having laid a certain number of eggs. To control the beetle more effectively with attractant, we need another chemical that attracts virgin or young females that have laid no or few eggs.

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Pteridine Accumulation for Age Determination in the Melon Fly, *Bactrocera (Zeugodacus) cucurbitae* (COQUILLET) (Diptera: Tephritidae)^{1,2}

Atsushi MOCHIZUKI,³ Masakazu SHIGA⁴
and Osamu IMURA⁴

³ Tohoku National Agricultural Experiment Station,
Yotsuya, Omagari, Akita 014-01, Japan

⁴ National Institute of Agro-Environmental Sciences,
Kannonnai 3-1-1, Tsukuba, Ibaraki 305, Japan

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The melon fly *Bactrocera cucurbitae* is a serious pest of various beneficial fruits, vegetables and trees South-East Asia and Hawaii. Since 1972, an eradication program of the melon fly with the sterile insect technique has been successfully conducted in subtropical region (the Nansei Islands) of Japan (NAKAMORI, 1991; SHIGA, 1991 a, b). Knowledge of age distribution of released flies as well as the wild fly population in the field is necessary for evaluating the effect of sterile insect release, quality of mass-produced flies, etc.

However, age determination of adult insects is difficult. MAIL et al. (1983) proposed a method for determining the age of *Stomoxys calcitrans* that depends on the accumulation of fluorescent materials in the head capsule with time. This method proved to be much more precise and simple than other methods such as changes in the reproductive

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Fig. 1. Relationship between pteridine fluorescence level and chronological age for adults of the melon fly at 27°C. The fitted equation using nonlinear least-square method was $y=12.24x^{0.170}$ ($N=30$, $p<0.001$) for the males (A) and $y=10.43x^{0.215}$ ($N=54$, $p<0.001$) for the females (B). Bars represent standard error.

systems (TYNDALE-BISCOE, 1984), and is efficient for prompt treatment of many samples. The method was applied for the age determination of several flies:

Glossina morsitans morsitans (LEHANE and MAIL, 1985), *Simulium damnosum* (CHEKE et al., 1987), *Glossina* spp. (LANGLEY et al., 1988), *Cochliomya hominivorax* (THOMAS and CHEN, 1989), *Chrysomya bezziana* (WALL et al., 1990) and *Lucilia sericata* (WALL et al., 1991). In this paper, we report pteridine accumulation in the melon fly and evaluate its usefulness in the determination of chronological age of the fly.

Larvae of the fly were reared on an artificial diet (NAKAMORI and KAKINOHANA, 1980) at 27°C with a natural day length in the mass production facilities of the Okinawa Prefectural Fruit Fly Eradication Office (KAKINOHANA, 1991). Adults were fed on water, sugar and protein hydrolysate. Adult flies were collected for analysis from the stock culture at one week intervals after emergence (from 2-days-old to 37-days-old) and freeze-killed at -20°C. The flies were placed in a cooler box at 0°C with silica gel and carried to the National Institute of Agro-Environmental Sciences, Tsukuba by airplane. The flies were stored at 5°C under a dark and dry condition before use. Pteridine was stable under this condition for 37 weeks (LEHANE and MAIL, 1985).

The flies were decapitated and their head widths were measured under a dissecting microscope with

an ocular micrometer. Pteridine was extracted by sonicating the head in a 1.5 ml micro tube with 200 μ l of solvent (water, methanol, acetonitrile, acetic acid; 90, 10, 0.5, 0.1 by volume) (NOBLE and WALKER, 1990) at 40 W for 15 s on ice with a Sonifier® Model 250 (Branson) with microchip accessory. The sonicated mixture was added with 800 μ l of the solvent, mixed well and centrifuged for 5 min at 15,000 rpm at 20°C. Fluorescence of 800 μ l of the supernatant was measured using a fluorescence spectrophotometer (Hitachi 650-60®) with excitation wavelength at 360 nm and emission wavelength at 450 nm. The excitation and emission slits were 2 mm and 10 mm, respectively. Five heads of the males and 8-10 heads of the females were analyzed for each age group.

The pteridine level in heads of both sexes increased with chronological age (Fig. 1). Relationship between the level of fluorescence (y) and the chronological age (in days) (x) was curvilinearly fitted ($y=ax^b$). The equation for males was $y=12.24x^{0.170}$ ($N=30$, $p<0.001$) and that for females was $y=10.43x^{0.215}$ ($N=54$, $p<0.001$). The curvilinear relationship was also reported in the old-world screw-worm fly, *Chrysomya bezziana* (WALL et al., 1990) and the blow-fly, *Lucilia sericata* (WALL et al., 1991). Although the differences in the slopes (b) between the two equations were not significant, the intercepts ($\ln$ of a) were significantly higher for males than for females ($p<0.05$). A similar

Fig. 2. Relationship between pteridine fluorescence level and relative head size (the cube of head width) of 16-day-old females. $N=24$, $r=0.62$ ($p<0.01$).

relationship, with a higher pteridine level of the intercepts for males than for females was observed in *G. austeni*, *G. morsitans morsitans* and *G. pallides* (LANGLEY et al., 1988).

WALL et al. (1991) reported that body size (wing vein length) of *L. sericata* was positively correlated with pteridine level but did not affect the accumulation rate. Although correlation between the cube of head width (mm³) of 16-day-old female flies and their fluorescence was significant ($N=24$, $r=0.62$, $P<0.01$) (Fig. 2), the regression explains 38% of variability in fluorescence. As THOMAS and CHEN (1989) suggested, the laboratory population might not have enough variation in the head capsule size to reflect its effect on variation in the pteridine level.

We concluded that the pteridine method might be applicable to determine the age of the melon fly. In the model proposed here, however, the precision of the estimated age decreased with time, because the variance of the fluorescence level increased with age. Further studies should be

made to correct fluorescence values for ambient or body temperature, which is known to improve age prediction slightly under laboratory conditions (LEHANE and MAIL, 1985; LANGLEY et al., 1988). Precision of the estimated age with this method should be also clarified for actual application (THOMAS and CHEN, 1989).

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