identical to that of the latex agglutination test of SHIMIZU and ARAKAWA (1986). For the agglutination test, 50 μ l of the virus suspension or silkworm larval extract was mixed with 50 μ l of IgG coupled protein-A-coated latex particles suspension on a slide glass. Agglutination was examined after shaking for 5 min. The degree of agglutination was graded as strongly positive with large aggregates, weakly positive with a visible agglutination and milky liquid, or no agglutination (see Table 1).

Fourth instar silkworm larvae were inoculated orally with polyhedra (NPV or CPV, 10^6 /ml) or distilled water as control. After 7 days, larvae were collected and homogenated. Silkworm larval extract was the supernatant of larval homogenates in 10-folds (w/v) of 0.1 M Tris-HCl buffer (pH 8.0) which had been centrifuged at 10,000 rpm for 5 min. Protein concentration was determined by the method of LOWRY et al. (1951).

In a series of experiments in which various concentrations of protein A and IgG were used to detect 2 μ g/ml of purified virions, a combination of 25 μ g protein-A/ml and 0.3 mg IgG/ml gave the best condition (Table 1). In this condition, the minimum detectable concentration of purified virions was 0.13 μ g/ml as compared with 1.0 μ g/ml in the ordinary latex agglutination test (Table 2).

NPV-infected silkworm larval extract was detected 8 times more sensitively by the protein-A-coated latex agglutination test (Table 3). No agglutination occurred when extract of CPV infected or uninfected larvae was used.

The results indicated that the sensitivity of protein-A-coated latex agglutination test for the detection of NPV was higher than that of latex agglutination test, and was easily adaptable to the diagnosis of the virus diseased larvae in domestic colonies of silkworm larvae.

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Effect of Relative Humidity during Pupal Maturation on Subsequent Adult Eclosion and Flight Capability of the Melon Fly, *Dacus cucurbitae* COQUILLETT (Diptera: Tephritidae)¹

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The laboratory environment is important in the artificial rearing of candidate insects for sterile insect release programs. In the Mediterranean fruit fly, *Ceratitis capitata* WIEDEMANN, the optimum

temperature and relative humidity for pupal development were determined by LANGLEY et al. (1972). In the melon fly, *Dacus cucurbitae* COQUILLETT, adult eclosion rates were adversely affected when pupal holding temperature was below 18°C or above 28°C (NAKAMORI and KAKINOHANA, 1980). The effect of relative humidity during the pupal period has not yet been investigated in the melon fly. This paper reports the effects of relative humidity in the pupal period on adult eclosion and flight capability of the melon fly.

MATERIALS AND METHODS

Melon flies were from a stock that had been reared for ca. 64 generations in the laboratory on an artificial diet (NAKAMORI and KAKINOHANA, 1980). About 6,000 mature larvae that had crawled out of the larval medium six days after egg seeding

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were placed in a plastic box (78 cm × 33 cm × 16 cm) containing a pupariation substrate consisting of sawdust, sand and water (1,000, 500 and 30 ml, respectively; moist pupariation substrate). The larvae were subjected to nine different holding regimens (I–IX) at ambient laboratory temperature (25°C) as shown in Fig. 1. Pupariation occurred within one day and adult eclosion took place 10 days after larvae were placed in the substrate.

In treatments I through VIII the pupae in the moist substrate were gently transferred from the plastic box to a screen-bottomed tray after 24 hr (1st day) and allowed to dry in ambient laboratory humidity (45–60% RH: low RH) for 24 hr (drying period) to facilitate separation from the substrate. Separated by gentle sieving, the pupae were thereafter held in open Petri dishes (9 cm dia.). In treatment IX, pupae were left in the original moist pupariation substrate until the 8th day, when sieving took place. In treatments III through VIII, a high humidity condition (80–90% RH: high RH) was created by placing the Petri dishes on a shelf in a plastic container (78 cm × 33 cm × 16 cm) containing water at the bottom. The container lid had six holes (8 mm dia.) to permit air exchange. The low humidity condition in treat-

ments I through VIII equaled the ambient laboratory humidity.

To determine the flight capability of the flies which were within two days after adult emergence, 100 pupae were placed in a Petri dish (9 cm dia.) on the eighth day (two days before eclosion). The Petri dish was surrounded by cardboard tubing (20 cm high) coated on the inside with talc that prevented flies from crawling out but permitted flying (BOLLER et al., 1981). To simulate high humidity in treatments II, III, IV, VIII, and IX during the flight capability test, folded paper tissues saturated with ca. 8 ml of water were placed close to the pupae. Humidity was monitored throughout the test period with a psychrometer and a hair hygrometer. The net percentage of fliers was calculated ((no. fliers/no. pupae examined) × 100). Each treatment was replicated five times. An analysis of variance was made after angular transformation of the data. Differences among means were assessed by DUNCAN's multiple range test. All experiments were performed during February and March 1982.

RESULTS AND DISCUSSION

The highest net rate of fliers was obtained from treatments VIII and IX, where the pupae were

Fig. 1. The various pupal holding regimens investigated.

Table 1. Rates of adult eclosion, fliers and net rate of fliers of *Dacus cucurbitae* pupae subjected to high and low relative humidities at different ages and for different durations

Treatments ^a	Adult eclosion (%)	Fliers (%)	Net % fliers ^b
I	22±2 a ^c	69±6 a	12±2 a
II	35±1 b	71±7 a	23±3 b
III	35±3 b	80±5 bcd	25±3 b
IV	46±3 c	81±4 cd	35±6 c
V	67±2 d	82±4 deh	50±2 d
VI	76±1 e	88±3 efh	63±3 e
VII	74±2 e	93±1 fgh	65±3 e
VIII	89±1 f	95±3 g	83±3 f
IX	95±1 g	89±3 h	83±4 f

^a See Fig. 1. ^b See text. ^c Values (mean±SE) in the same column followed by the same letter are not significantly different from each other at the 5% level (DUNCAN'S Multiple Range Test).

not subjected to low RH (Table 1). When the pupae were kept at high RH the net flier rate was not affected even though they were separated from the pupariation substrate two days after the onset of rearing. On the other hand, the pupae obtained from treatment I, where the pupal holding time in low RH was the longest, showed the lowest net rate of fliers. In treatments I to IV (the first half of the pupal period), or V to VIII (the latter half of the pupal period) the net flier rate increased as the pupae were held in high RH longer. When pupae were exposed to low RH for the same durations (i.e. treatments II and V, III and VI, and IV and VII), earlier initiation of exposure to the low RH led to lower net flier rates. The net flier rate of treatment IV was significantly lower than those of treatments V and VI, although the holding time in low RH was shorter in the former. These results show that the low RH was more harmful to the net flier rate at younger pupal ages than older ones, even though the pupal holding duration in low RH was shorter. The net flier rate seems to reflect the rate of adult eclosion (Table 1).

The optimal relative humidity for *C. capitata* pupae is 75% to 90% (LANGLEY et al., 1972). Although the minimum permissible RH for main-

taining high standards of adult eclosion and flight capability was not determined in this investigation, we could nevertheless conclude that a damp condition for holding pupae (80–90% RH) that is maintained as long as possible (or at least through the first half of the pupal period) is of critical importance to the quality of the flies.

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