Fig. 3. Daily changes in the number of stings by *Encarsia* sp. females on healthy or parasitized pupae of the greenhouse whitefly.

It is probable that success in matings by *Encarsia* sp. males at the emergence site depends on finding stationary virgin females shortly after emergence. This is because newly emerged females of *Encarsia* sp. stood still at a point about 2 cm away from the host for about 30 min after emergence.

In the present study, no aggressive interaction among males was observed. Once copulation occurred, mated females lost sexual receptivity to males. There was probably a competitive advantage to males which reached virgin females at the emergence site.

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Control Methods for *Anoplophora malasiaca* THOMSON (Coleoptera: Cerambycidae) in Citrus Groves.

I. Comparison of Effects in Several Methods for Preventing Oviposition^{1,2}

Ishizue ADACHI and Ryoji KORENAGA

Okitsu Branch, Fruit Tree Research Station, MAFF
 Okitsu, Shimizu, Shizuoka 424-02, Japan

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The white-spotted longicorn beetle, *Anoplophora malasiaca* THOMSON, is the most dreaded wood-infesting pest of citrus trees. In Shizuoka Prefecture, adults emerge from late May to late July. Adult females live for more than 2 months. They lay about 200 eggs in their lifespans, mainly at the bottom of the trunk of the citrus tree (ADACHI, 1988). Larvae destroy the cambium of the living trunk in their early developmental stages and the xylem in late stages, which causes tree deterioration

or death. In recent years, injuries by this insect are increasing on citrus in Japan.

Although controls for *A. malasiaca* have been carried out by killing adults, eggs and larvae, and protecting trees from its oviposition (KAWAMURA, 1976; KAWAMURA and YAMAMOTO, 1980), no effective control method fully suppressing the damage by this species has been established (KATO, 1982; KASHIO, 1982). In this paper, we compared the effects of several oviposition-preventing methods, as a step in constructing an IPM system.

MATERIALS AND METHODS

The study was conducted in 1987 at the Oya Experimental Grove of the Fruit Tree Research Station in the central part of Shizuoka Prefecture. Five to ten year-old citrus trees were used to compare the effects of the following 5 treatments preventing *A. malasiaca* oviposition: (TR-1) spraying whitewash including organophosphorous insecticide (fenitrothion 15%) to the bottoms of trunks

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(39 trees), (TR-2) loosely covering the bottoms of trunks with externally sticky cardboard 11 cm in width (41 trees), (TR-3) covering the bottoms of trunks threefold with 2 cm mesh fishing net 22 cm in width (41 trees), (TR-4) covering the bottoms of trunks with fine-meshed (6 openings/cm) wire net 13 cm in width (41 trees), and (TR-5) no treatment (36 trees). In TR-4, soil was piled at the bases of the trunks below the nets (Fig. 1). These 5 treatments were conducted randomly throughout the grove.

Treatments were applied on June 1 and 5, except for TR-1, which was applied on June 19. After June 19, *A. malasiaca* eggs were taken out from the trunks with tweezers and counted at almost weekly intervals.

RESULTS

Figure 2 shows seasonal changes in average number of eggs laid per tree in each treatment. Total numbers of eggs laid per tree throughout the study and their 95% confidence limits in TR-1 to TR-5 were 2.667 ± 1.047 , 1.463 ± 0.941 , 2.171 ± 0.879 , 0.244 ± 0.263 and 3.167 ± 1.188 , respectively. The number of eggs in TR-4 (wire net treatment) was relatively small, indicating that this treatment was most effective in preventing *A. malasiaca* oviposition. All of the eggs in TR-4 were laid on the uncovered parts of the trunks situated above the parts covered with the wire netting.

Figure 3 shows seasonal and cumulative changes of the number of trees oviposited by *A. malasiaca*.

Fig. 1. Schematic drawing of the treatment using fine-meshed wire net.

The ranking of oviposition rates of the treatment groups, i.e., number of oviposited trees/total number of trees, was $TR-5 \cong TR-1 > TR-3 > TR-2 \gg TR-4$. Thus, *A. malasiaca* oviposition was most effectively obstructed by the wire net treatment.

TR-2 (sticky cardboard treatment) and TR-3 (fishing net treatment) serve the purposes of capturing *A. malasiaca* adults as well as preventing oviposition. The number of adults captured in these treatments during the study was 0 in TR-2, and 17 (1 male and 16 females) in TR-3.

DISCUSSION

It is difficult to assess the relationship between the number of larvae infesting trees and the degree of damage caused by those larvae. However, even a small number of larvae can easily kill young citrus trees and defoliate old ones. Therefore, the economic injury level (EIL) should be set at a low infestation rate. This condition may be attained by

Fig. 2. Seasonal changes in the average number of eggs laid per tree treated with (A) fine-meshed wire net (TR-4), (B) cardboard (TR-2), (C) fishing net (TR-3), (D) whitewash (TR-1), and (E) no treatment (TR-5). Vertical bars represent 95% confidence limits. Numerals on the upper right of each small figure are the total numbers of eggs per tree and 95% confidence limits.

Fig. 3. Seasonal (●) and cumulative (○) changes in the number of trees oviposited by *A. malasiaca*. The trees had undergone the following treatments; (A) fine-meshed wire net (TR-4), (B) cardboard (TR-2), (C) fishing net (TR-3), (D) whitewash (TR-1), and (E) no treatment (TR-5). Numerals in the figure indicate the numbers of trees oviposited at least once during the study. The ratios are in parentheses.

using "preventive" control methods to protect trees from oviposition, as well as the "expulsive" methods of killing adults, larvae and eggs with insecticides, etc.

The spraying of insecticide and the capture of adults in citrus groves by hand are exclusion methods that have been used, but do not have much effect in reducing adult populations (KOMAZAKI and SAKAGAMI, 1988; ADACHI, unpublished). KATO (1982) also reported that adult density in Oshima Island, Yamaguchi Prefecture, had stayed high for several years in spite of vigorous exclusion efforts. A study on population dynamics using marking and recapture hypothesized that adults frequently moved in and out of citrus groves (ADACHI, unpublished). This frequent movement may be one of the reasons for the difficulty of suppressing adult density in citrus groves.

A. malasiaca eggs are laid repetitively during a

3 month period and larvae live under bark. Periodical and attentive control actions are indispensable to detect them early enough and to check injuries by larval feeding. Exclusion methods (insecticide spraying and killing adults by hand) therefore require high labor hours/costs to achieve high control efficiency.

Continually protecting oviposition sites in citrus trees, i.e., mainly the lower parts of the trunks, would reduce costs and labor. To prevent *A. malasiaca* oviposition, the following two methods can be utilized: repelling adult females from citrus trees and eliminating oviposition sites for females. With the former method, painting whitewash on the bark of citrus trunks has been tried. KAWAMURA (1976) clarified that the whitewash method was not effective. The present study also showed a similar result. For the latter method, citrus trunks have been covered with physical substances. For example, KOBAYASHI (1957) used slaked lime hardened with polyvinyl alcohol and polyvinyl acetate emulsion; KAWAMURA (1976) used newspaper. These methods were highly effective in preventing oviposition. In the present study, using wire net was also shown to be very effective. Methods covering oviposition sites with physical substances are very effective and should be applied as an elemental control strategy in an integrated pest management (IPM) system for *A. malasiaca*.

Desirable attributes of such "physical protection" methods are high certainty to prevent oviposition, long continuity of effect, ease of setting, and low cost, etc. With the fishing net method, certainty seems to be low, because females can lay eggs through the mesh. With the cardboard method, the trunk surfaces became too moist, resulting in bark rot. KOBAYASHI's and KAWAMURA's methods lack continuity of effects. In contrast, the wire net method does not seem to have any similar defects. In the future, it will be necessary to examine the combination of this wire net method and the other materials which allow the capture of adults (such as fishing net).

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Infection of a *Bombyx* Cell Line with a Cytoplasmic Polyhedrosis Virus of the White Cutworm, *Euxoa scandens* (Lepidoptera: Noctuidae)¹

Hajime INOUE and Serge BELLONCIK²

National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305, Japan

²Centre de Recherche en Virologie, Institut Armand-Frappier, Université du Québec, Laval, Qc, Canada H7N4Z3

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The larval midgut is the target of insect pathogenic cytoplasmic polyhedrosis viruses (CPVs), however, several established cell lines derived from ovarian tissues have supported the replication of CPVs (GRANADOS et al., 1974; QUIOT and BELLONCIK, 1977; LONGWORTH, 1981). In the present study, we examined the infection of a CPV of the white cutworm *Euxoa scandens* on a cell line (SES-BoMo-15A) derived from the embryos of the silkworm *Bombyx mori* (INOUE and MITSUHASHI, 1988).

A freeze-dried sample of *E. Scandens* CPV, which was obtained from the filtration of a homogenate of one larval midgut, was resuspended with 1 ml of MGM-448 medium (MITSUHASHI, 1984), and diluted 10 times before using as an inoculum. The cells (about 3×10^5 /ml) were pelleted on day 2 following subculture by centrifugation at 200 *g* for 2 min and resuspended with 1 ml of virus suspension in a centrifuge tube. The tube was placed horizontally for 1 hr and rocked several times at 15 min intervals. The cells were transferred into a two-chamber culture slide (Lab-Tek, Miles Inc.) at 0.5 ml per chamber, and after the addition of 1 ml

of fresh medium, incubated at 25 °C.

On day 4 post virus inoculation, tetragonal crystals appeared in the cell cytoplasm (Fig. 1a). With time, the shapes of the crystals became more clear and number of crystals increased. Almost all crystals were tetragonal; a few hexagonal crystals were present (Fig. 1b). When the culture slides were treated with 1% bromphenol blue (BPB) after pretreatment with methanol, followed with boiling water as described by ABE (1973), the crystals were well stained, revealing that they were the polyhedra of CPV (Fig. 1c). The formation of polyhedra was the same as those of the CPVs of *Tricoplusia ni*, *Chrysodeixis exiosoma*, and *E. scandens*, which mainly formed cuboidal polyhedra *in vitro* in contrast to the spherical polyhedra produced *in vivo* (GRANADOS et al., 1974; BELLONCIK and BELLE-MARE, 1980; LONGWORTH, 1981).

Most cells of the *B. mori* cell line were of the floating type, but some cells were attached to the culture slides. Since polyhedra were frequently observed in cells attached to culture slides, the infection rates of the two types of cells, attached and floating, were examined. On day 7 post CPV inoculation, the floating cells were collected, washed with CARLSON's salt solution by low speed centrifugation and smeared on glass slides. These glass slides, and the slides prepared from attached cells which also had been rinsed with CARLSON's solution, were stained with BPB as described above. Cells containing polyhedra were counted. The attached cells produced much more polyhedra than floating cells (Table 1). The number of polyhedra per attached cell ranged from 4 to 51 with an average of 20.4.

Using electron microscopic observation, the attached cells were fixed with 2.5% glutaraldehyde and 1% osmium solutions, and dehydrated with

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