one tenth of the infestation level in 1983. For five years in the 1960's (before the introduction of *T. sinensis*), *D. kuriphilus* population density evaluated based on numbers of galls showed approximately fourfold population fluctuations (Miyashita et al., 1965). It is believed, therefore, that the rapid decrease in infestation observed in the present study is largely due to the single release of *T. sinensis* at FTRS.

A tolerable injury level by *D. kuriphilus* for the chestnut tree is reported as 30% infestation of current shoots (Gyoutoku and Uemura, 1985). This means that the chestnut trees at FTRS were more or less adversely influenced by the *D. kuriphilus* infestation until 1985 (Fig. 1). In 1986, however, the situation drastically changed. The infestation level became much lower than the tolerable injury level. Currently, it has become almost impossible to find a tree bearing hundreds of galls at FTRS.

Recently *T. sinensis* adults have been obtained from galls collected out of FTRS. Fortunately, adults of this species appear in spring, the season when no chemical spray program is practiced in the chestnut orchards. Therefore, it is concluded that the result of the introduction of *T. sinensis* as a biological control agent is very successful as a means of controlling the chestnut gall wasp in Japan.

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A First Record of the Entomopathogenic Fungus, *Neozygites parvispora* (MacLeod & Carl) Rem. & Kell., on *Thrips palmi* Karny (Thysanoptera: Thripidae) in Japan

Tsutomu Saito, Sakae Kubota,
and Mitsuki Shimazu

Shizuoka Agricultural Experiment Station,
Iwata, Shizuoka 438, Japan

Forestry and Forest Products Research Institute,
Tsukuba, Ibaraki 305, Japan

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*Thrips palmi* Karny has become an important pest of melon, cucumber, eggplant, chrysanthemum and many other crops since being introduced into Japan in the late 1970's. In October, 1987, an entomophthoralean fungus was found infecting this thrips on glasshouse-grown melons (variety: Earl's favourite) at the Shizuoka Agricultural Experiment Station. Based on its morphological characteristics, this fungus was identified as *Neozygites parvispora* (=*Entomophthora parvispora*). The fungus was first found on *Thrips* spp. (principally *T. tabaci* Lind.) by Carl (1975). Subsequently MacLeod et al. (1976) described it as *Entomophthora parvispora* and since then it has been found in Europe (MacLeod et al., 1976; Ramakers, 1978; Samson et al., 1979; Mietkiewski and Geest, 1985), the USSR (Tsintsadze et al., 1984) and South America (Aruta et al. 1984). Prior to this note the fungus was not known in Japan. The nomenclature used here is as in Remaudière and Keller (1980).

Larvae killed by the fungus became light yellow in colour, and their surfaces appeared velvety as conidiophores of *N. parvispora* penetrated the entire integument of the insect. In adult diseased thrips the fungus grew throughout the intersegmental membranes (Fig. 1a). Conidiophores are simple, unbranched and produce spherical to short-ovoid, slightly smoke-coloured conidia with broad or rounded papillate bases (Fig. 1b). Conidial size ranged from 12.5–15.0 μm × 9.3–11.3 μm and

2 Present address: Shizuoka Prefectural Citrus Experiment Station, Komagoe, Shimizu 424, Japan
averaged $13.6 \pm 0.8 \mu m \times 10.2 \pm 0.3 \mu m$ (mean ± standard deviation). These conidia were discharged short distances from cadavers. Primary conidia produce almond-shaped capitlolospores with a discoidal structure (Fig. 1c) on long (48.1 ± 4.5 μm) and thin capillary conidiophores; their size varied from 10.8–17.5 μm × 7.0–9.5 μm, the average being $14.2 \pm 1.7 \mu m \times 7.7 \pm 0.5 \mu m$. MacLeod et al. (1976) reported that the fungus did not possess primary-type anadhesive spores, but we rarely observed replicative conidia (Fig. 1d) similar to primary conidia. Cystidia and rhizoids were not observed. These morphological characteristics are similar to those of *N. parvispora* (MacLeod et al., 1976), except for the production of primary-type anadhesive spores. Resting spores (zygospores), that normally form towards the end of season (Carl, 1975), were not observed.

In the glasshouse about 10% of the thrips (adults and larvae) were continuously attacked by the fungus until the melon harvest in late November. Mature larvae drop from melon leaves before they pupate, so affected pupae are likely to die on the ground. Diseased *T. palmi* were evident on the surface of leaves. The fungus did not provide satisfactory control of *T. palmi*, because melon leaves were severely damaged by the many thrips that survived. Attempts to cultivate the fungus from diseased thrips, using Sabouraud glucose agar amended with yeast extract and egg yolk, failed.

REFERENCES

Titration of *Bombyx mori* (Lepidoptera: Bombycidae) Nuclear Polyhedrosis Virus in a *Bombyx mori* Cell Line\(^1\)\(^2\)

Toshihiro Nagamine, Miyako Shimomura, Hideaki Sugimori and Michihiro Koyamash

*Laboratory of Sociocultural Science, Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan*

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Since Hink and Vali (1973) demonstrated the plaque-forming ability of *Autographa californica* nuclear polyhedrosis virus (AcNPV) in a continuous cell line from *Trichoplusia ni*, several investigators have developed and established the plaque assay technique for various species of NPV (cf., Hughes and Wool, 1986). The plaque assay serves as the most reliable and versatile assay system in animal virology, and provides not only a precise and reproducible titration method for virus infectivity but also a means for the isolation of genetically homogeneous virus stocks. Establishment of the plaque assay technique is thus an essential prerequisite for the detailed fundamental research on the replication mechanism of the virus.

Recently the plaque assay technique has also been successfully applied to *Bombyx mori* (Bm) NPV carried by a continuous cell line (Maeda, 1984), and some elegant molecular engineering works have been carried out (Maeda et al., 1984, 1985). Systematic studies correlating the virus growth cycle with biochemical or cytological events occurring in the infected cells have not been conducted. As the first step of these plaque assay technical studies, we isolated virus clones from the hemolymph of BmNPV-infected *Bombyx* larvae and defined the growth kinetics of the virus released from the cultured cells infected with a cloned BmNPV. In addition, the sensitivity of plaque assay in the titration of virus infectivity was compared with those of quantal assays using living animals and cultured cells.

The continuous cell line, BM-N, derived from *Bombyx* (cf., Volkman and Goldsmith, 1982) was used in these experiments. These cells were maintained at 28°C in BML-TC10 medium (Gardiner and Stockdale, 1973) supplemented with 10% fetal calf serum (Watanabe, 1987). The BmNPV used for the virus cloning was isolated from naturally infected *Bombyx* larvae (Koyamash and Nakagaki, 1984), and initial inoculum for plaque production was derived from the hemolymph of the infected larvae. The hemolymph collected was centrifuged at 12,000 g for 15 min at 4°C and resultant supernatant was filtered through a 45-μm Millipore filter. The filtrate was diluted with BML-TC10 medium at desired concentrations before use.

The plaque assay was performed as described previously (Maeda, 1984) with some modifications. The BM-N cells (5 × 10⁶) were seeded in 60-mm plastic Petri dishes and allowed to attach for 1–2 hr. The medium was removed and 0.5 ml of virus inoculum was applied to the monolayer culture. After a 1-hr adsorption period, the inoculum was replaced by 3 ml of overlay medium containing 0.5%, Seapaque agarose (FMC Co.) in BML-TC10 medium. After a 20–30 hr incubation at 28°C, 3 ml of overlay medium was again added to each plate to ensure the nutritional supply and the plates were incubated at the same temperature for 5–6 days until the plaques became visible.

For virus cloning, plaques were removed into each 0.5 ml of BML-TC10 medium by Pasteur pipettes and virus suspensions were re-plated on the BM-N cell monolayers after appropriate dilutions. This plaque-purification procedure was repeated three times before the BmNPV clone was obtained. Working stocks of the cloned virus were prepared

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