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Letter to the Editor

VIRUS SUSCEPTIBILITIES OF NEW CELL LINES FROM EMBRYOS OF THE WHITEMARKED TUSSOCK MOTH

Dear Editor:

The whitemarked tussock moth, *Orgyia leucostigma*, is an occasional pest of deciduous trees in North America. While usually not as serious as the gypsy moth or a related species, the Douglas fir tussock moth, it is part of a complex of insect pests which can occur in damaging numbers. A number of pathogens that are effective in controlling these pests have been identified, including a homologous nuclear polyhedrosis virus (NPV) and cytoplasmic polyhedrosis virus (CPV).

Previous reports described the establishment of cell lines from neonate larvae (Sohi et al., 1981) and the replication of *O. leucostigma* NPV (OlNPV) in several of these (Sohi et al., 1984). In our lab, we have used embryonic tissues to establish several new cell lines from various insects (Rochford et al., 1984; Lynn et al., 1988) which were found to be more susceptible or better producers of some NPVs (Lynn et al., 1989; Lynn, 1992). In the current study, we used embryos of the whitemarked tussock moth to initiate cultures. Three continuous cell lines have been established and their susceptibility to various viruses has been determined.

Eggs of *O. leucostigma* were obtained from Dr. J. C. Cunningham, (Forest Canada, Sault Ste. Marie, Ontario) and the insects were reared on standard gypsy moth diet (Bell et al., 1981). Primary cultures were set up as previously described (Lynn, 1989). Briefly, egg masses were disinfected and approximately 50 eggs were cut open with a microscalpel. The released embryos were rinsed in 1 ml modified TC-100 (Lynn, 1989) supplemented with gentamicin sulfate (50 μg/ml; Sigma Chemical Co., St. Louis, MO) before being minced in a 0.15-ml standing drop of medium. Medium was added or replaced at 7–10 d intervals until cells could be subcultured. Initial subcultures were performed by flushing with medium from a pipette to loosen cells and transferring contents with additional fresh medium into a 25-cm² tissue culture flask (Falcon®).[1] Two of the original primary cultures resulted in continuous growth. One of these (designated IPLB-OlE505) had distinctly different populations of attached and suspended cell types. A selection process was initiated at the second passage in which we subcultured the suspended cells by removing an aliquot of the medium from the culture and mixing it with fresh medium in a new flask. This became the suspended strain, IPLB-OlE505s (Fig. 1 *A*) which was maintained by transfer of only suspended cells at each passage. The second passage culture was then rapped firmly and the medium removed. The culture was rinsed with three changes of medium and returned to the incubator. This procedure was repeated on the second passage culture for sev-

[1]Mention of proprietary or brand names is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

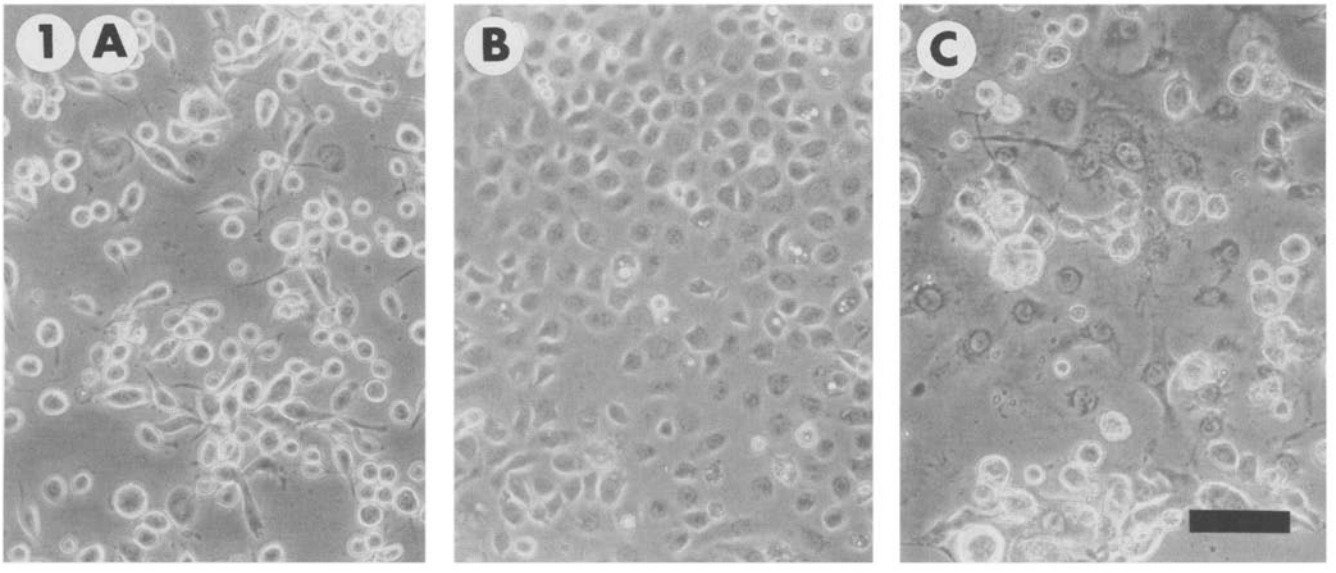

FIG. 1. Phase-contrast photomicrographs of (*A*) IPLB-OlE505s, (*B*) IPLB-OlE505A, and (*C*) IPLB-OlE7. All micrographs are at the same magnification; *marker bar* = 50 μm.

eral weeks until a confluent monolayer of firmly attached cells was present in the culture after rinsing. At this point, we subcultured cells by rinsing with trypsin buffer (8.15 g NaCl, 0.2 g KH_2PO_4, 0.2 g KCl, 1.5 g Na_2HPO_4, and 0.23 g Na_2EDTA per liter demineralized H_2O) and then trypsinizing for 30 min with 0.05 mg VMF trypsin (Worthington Biochemical Corp., Freehold, NJ) per ml trypsin buffer. This was recorded as the third passage of the IPLB-OlE505A strain (Fig. 1 *B*). We continued the selection pressure for attached cells on the third and all subsequent passages in the same manner as above by firmly rapping to remove loose cells and rinsing with fresh medium each week. The third strain of *O. leucostigma* cells was established from a separate primary culture and was designated IPLB-OlE-7. It consisted of a mixture of suspended and attached cells which were larger than those of the OlE505 strains (Fig. 1 *C*).

The three new strains were characterized by isozyme analysis with the AuthentiKit™ system. Our usual protocol uses the enzymes which were proposed by Tabachnick and Knudson (1980) as adequate for separating all the insect cell lines they had tested: phosphoglucose isomerase (PGI), phosphoglucose mutase (PGM), malic enzyme (ME), and isocitrate dehydrogenase (ICD). For the first time since we began using this procedure, these enzymes were not adequate for discriminating the new *O. leucostigma* lines from another line maintained in our laboratory (IPLB-SF21AE from *Spodoptera frugiperda*, Vaughn et al., 1977). Only ICD showed a slightly different pattern in which one band in IPLB-SF21AE did not occur in the *O. leucostigma* lines. However, with additional enzyme analyses with aspartate aminotransferase (AST), peptidase B (PepB), and malate dehydrogenase (MD), differences became apparent. The patterns exhibited for each of these enzymes in the *O. leucostigma* lines differed from the pattern seen with IPLB-SF21AE (data not shown). Between these additional enzyme systems and the original four used, we could differentiate the new lines from all the other cultures currently maintained in our laboratory.

We performed virus studies by initiating cultures in 24-well tissue culture plates (Corning™) in 1.0 ml modified TC-100 supplemented with gentamicin sulfate (50 μg/ml). Cells were then inoculated with 0.1 ml extracellular virus of the NPVs from *O. leucostigma* (Hughes, 1976; obtained from Dr. J. C. Cunningham), *O. pseudotsugata* (OpNPV, Hughes and Addison, 1970; obtained from Dr. J. C. Cunningham), *Lymantria dispar* (LdNPV, Shapiro et al., 1992), *Autographa californica* (AcNPV, Vail and Jay, 1973), *Rachoplusia ou* (RoNPV; Lewis and Adams, 1979), or *Anagrapha falcifera* (AfNPV, Hostetter and Puttler, 1991; obtained from Dr. P. Vail, USDA, Fresno, CA), or the CPV from *O. leucostigma* (isolated in our laboratory from the OlNPV sample, unpublished). From these tests, we found that all three lines were susceptible to OlNPV, OpNPV and OlCPV with complete formation of occlusion bodies. The noctuid viruses (AcNPV, RoNPV and AfNPV) produced occlusion bodies in the OlE505A strain but caused no discernible pathology in the other two lines. LdNPV caused no pathology in any of the lines. In addition to these tests, the IPLB-OlE505s strain was also inoculated with CPVs from *Trichoplusia ni* (TnCPV) and *L. dispar* (LdCPV) (both isolated in our laboratory, unpublished). No cytopathology was exhibited by TnCPV, but occlusion bodies were formed after inoculation with LdCPV.

We have described three new cell lines from the whitemarked tussock moth which are susceptible to the homologous NPV and CPV. One of the lines also replicates a CPV from *L. dispar* but not the gypsy moth NPV. Only the attached strain (OlE505A) replicated the three NPVs from noctuids which were tested. The new cell lines have different characteristics of cell growth which may make them each useful for various virus techniques. For example, the tightly attached IPLB-OlE505A may be useful in plaque assays and for plaque-purifying virus. The IPLB-OlE505s grows in suspension and at a more rapid rate and thus may be useful in production schemes. We are continuing the virus studies on these lines to determine their utility in these various procedures.

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References

Bell, R. A.; Owens, C. D.; Shapiro, M., et al. Development of mass rearing technology. Doane, C. C.; McManus, M. L., ed. The gypsy moth: research towards integrated pest management: U.S. Dep. Agric. Tech. Bull. #1584. 599–633; 1981.

Hostetter, D. L.; Puttler, B. A new broad host spectrum nuclear polyhedrosis virus isolated from a celery looper. *Anagrapha falcifera* (Kirby). (Lepidoptera, Noctuidae). Environ. Entomol. 20:1480–1488; 1991.

Hughes, K. M. Notes on the nuclear polyhedrosis viruses of tussock moths of the genus *Orgyia* (Lepidoptera). Can. Entomol. 108:479–484; 1976.

Hughes, K. M.; Addison, R. B. Two nuclear polyhedrosis viruses of the Douglas-fir tussock moth. J. Invertebr. Pathol. 16:196–204; 1970.

Lewis, L. C.; Adams, J. R. Pathogenicity of a nuclear polyhedrosis virus from *Rachoplusia ou* to *Agrotis ipsilon*. J. Invertebr. Pathol. 33:253–256; 1979.

Lynn, D. E. Methods for the development of cell lines from insects. J. Tissue Culture Methods 12:23–29; 1989.

Lynn, D. E. Improved efficiency in determining the titer of the *Autographa californica* baculovirus nonoccluded virus. BioTechniques 13:282–285; 1992.

Lynn, D. E.; Dougherty, E. M.; McClintock, J. T., et al. Development of cell lines from various tissues of Lepidoptera. Kuroda, Y.; Kurstak, E.; Maramorosch, K., ed. Invertebrate and fish tissue culture. Tokyo: Japan Scientific Societies Press; 1988:239–242.

Lynn, D. E.; Dougherty, E. M.; McClintock, J. T., et al. Comparative replication of *Lymantria dispar* nuclear polyhedrosis virus strains in three continuous-culture cell lines. Appl. Environ. Microbiol. 55:1049–1051; 1989.

Rochford, R.; Dougherty, E. M.; Lynn, D. E. Establishment of a cell line from embryos of the cabbage looper. *Trichoplusia ni* (L.). In Vitro 20:823–825; 1984.

Shapiro, M.; Lynn, D. E.; Dougherty, E. M. More virulent biotype isolated from wild-type virus. U.S. *Patent* No. 5132220. 1992.

Sohi, S. S.; Percy, J.; Arif, B. M., et al. Replication and serial passage of a singly enveloped baculovirus of *Orgyia leucostigma* in homologous cell lines. Intervirology 21:50–60; 1984.

Sohi, S. S.; Percy, J.; Cunningham, J. C., et al. Replication and serial passage of a multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae) in continuous insect cell lines. Can. J. Microbiol. 27:1133–1139; 1981.

Tabachnick, W. J.; Knudson, D. L. Characterization of invertebrate cell lines. II. Isozyme analyses employing starch gel electrophoresis. In Vitro 16:392–398; 1980.

Vail, P. V.; Jay, D. L. Pathology of a nuclear polyhedrosis virus of the alfalfa looper in alternate hosts. J. Invertebr. Pathol. 21:198–204; 1973.

Vaughn, J. L.; Goodwin, R. H.; Tompkins, G. J., et al. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In Vitro 13:213–217; 1977.

D. E. Lynn M. Shapiro

USDA, Agricultural Research Service
Insect Biocontrol Laboratory
BARC-West, Building 011A, Room 214
Beltsville, Maryland 20705-2350

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