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Development of a dipstick immunoassay to detect nucleopolyhedroviruses in Douglas-fir tussock moth larvae

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Abstract

In this paper, we describe the development of a novel field detection system for the identification of *Orgyia pseudotsugata* nucleopolyhedrovirus (OpNPV) and OpNPV infections in Douglas-fir tussock moth (*O. pseudotsugata*) (DFTM) larvae, utilizing antibodies in a dipstick immunoassay. The dipstick method is sensitive to a minimum of 10 ng of extracted virus protein, or approximately 1070 virus occlusion bodies, and is sufficiently sensitive to detect OpNPV infections in DFTM prior to mortality. Additionally, the method can be used to unambiguously detect virus in infected larvae without purification of the test sample. This research provides a novel tool for on-site assessment of the incidence of OpNPV in field populations of DFTM, and has the potential to improve the biological control of the DFTM by facilitating on-site pest management decisions.

Keywords: Baculoviridae; Nucleopolyhedrovirus; Immunodetection; Douglas-fir tussock moth

1. Introduction

The Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae) (McDunnough) (DFTM) reaches outbreak levels periodically, every 10–13 years, between the coastal and central mountain ranges in western North America (Stoszek and Mika, 1978; Harris et al., 1985; Myers, 1988; Mason et al., 1998). In its northern range, in British Columbia, foliage feeding by outbreak populations may lead to top kill and mortality of Douglas-fir trees, *Pseudotsuga menziesii* var. *glauca* (Beissn.)Franco (Alfaro et al., 1987). From southern Washington State to New Mexico, the DFTM predominantly consumes true fir species, *Abies* spp. Miller, and outbreak populations may cause tree mortality (Wickman, 1978).

The *Baculoviridae* is a family of insect-specific viruses that consist of enveloped virions containing large, double-stranded DNA genomes. Two genera, *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV), are distinguished on the basis of capsid size, shape and morphology (Jehle et al., 2006). The population densities of many forest defoliating insects are influenced by pathogenic NPVs or GVs (Stairs, 1972; Fuxa, 1987; Tanada and Fuxa, 1987; Myers, 1988; Moscardi, 1999), and the incidence of NPVs and GVs may be used to predict host population densities because these pathogens produce lethal infections that result in population decline (Anderson and May, 1981).

DFTM populations are modulated by naturally occurring, host-specific NPV pathogens, the *O. pseudotsugata* single NPV (OpSNPV) and *O. pseudotsugata* multiple NPV (OpMNPV) (Evenden and Jost, 1947; Steinhaus, 1951). Moreover, population densities of the DFTM can be predicted from the incidence of these NPVs in larvae raised from egg masses (Stelzer, 1979). An integrated pest management (IPM) program was developed to suppress outbreak populations of the DFTM. Moth populations are monitored and naturally occurring (laboratory propagated) OpMNPV may be introduced to suppress DFTM populations and prevent significant defoliation (Shepherd et al., 1984; Otvos and Shepherd, 1991; Otvos et al., 1987). Measurement of the incidence of OpNPVs within field larvae is integral to this program.

Currently, the incidence of OpNPV infections in DFTM populations is determined by microscopic analysis of macerated field-collected larvae or larvae reared from field-collected

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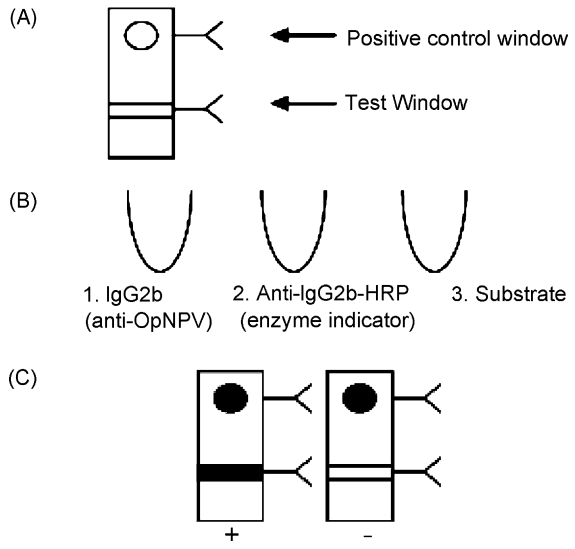

Fig. 1. Schematic diagram of the dipstick immunoassay for OpNPV detection. (A) Prepared dipsticks are sensitizing to a test sample for 15 min. (B) Sensitized membranes are incubated in: (1) mouse IgG2b OpNPV-specific antibody, (2) HRP-labelled anti-IgG2b antibody and (3) diaminobenzidine substrate for 15 min each. (C) True positive (double positive) results contain a clear brown line and circle. True negative results contain a clear brown circle at the control window. Permanent records are prepared by washing membranes three times in sterile ddH_2O. Dipsticks may be scanned and OB number estimated using a standard curve between luminosity^{-1} and OB number.

DFTM egg masses (Stelzer, 1979; Otvos et al., 1999). When the incidence of disease is low (<25%), augmentation of virus by aerial application may be conducted to prevent further defoliation (Stelzer, 1979). When the incidence is high (>25%), the natural incidence of the virus is considered sufficient to induce a viral epizootic and subsequent collapse of the pest population (Stelzer, 1979). The current method (microscopic analysis) is time consuming, and quantification is inaccurate below 1.0 million virus occlusion bodies (OBs) (Kaupp and Ebling, 1993). Thus, the development of a sensitive, on-site field detection system for OpNPV may be useful for on-site evaluation of OpNPV incidence and subsequent pest management decisions.

Rapid dipstick immunoassays (Fig. 1) have been shown to be effective tools for diagnosis of various diseases (Glad and Grubb, 1978; Horton et al., 1991; Snowden and Hommel, 1991; Rossi et al., 1991; Nataraju et al., 1994; Zhu et al., 2002). Previous work in this field has shown that dipstick immunoassays are viable for identifying baculoviruses (Nataraju et al., 1994), although this study did not use unpurified samples or field samples. James and Mukerji (1996) demonstrated that the cherry mottle leaf virus could be identified from crude plant extracts, but immunodiagnosis was not rapid. Rossi et al. (1991) developed and demonstrated that a rapid dipstick immunoassay was capable of detecting schistosomiasis and demonstrated that diagnosis could be made from whole blood. Their study demonstrated that rapid dipstick immunoassays could be used for field-based studies or low-technology laboratories with minimal manipulation of the test substrate.

The dipstick immunoassay pioneered by Glad and Grubb (1978) has since been modified to incorporate dye-labelled antibodies (Snowden and Hommel, 1991; Birnbaum et al., 1992; Nataraju et al., 1994) or colloidal gold- or silver-labelled antibodies (Horton et al., 1991), reducing the time of the assay. Nataraju et al. (1994) found that enzyme-labelled antibodies were more sensitive than dye-labelled antibodies. Shuy et al. (2002) reported that even with silver enhancement, gold-labelled antibodies were less sensitive than enzyme-labelled antibodies in immunodiagnostic tools. Although enzyme-labelled antibodies are more sensitive than other visual tags, they are not as efficient for rapid immunodiagnosis because of additional incubation steps (Paek et al., 2000).

In this paper, we described the development and use of a simple dipstick immunodiagnostic tool to detect OpMNPV OBs and OpMNPV infections in DFTM larvae. This is the first report that demonstrates rapid immunodiagnosis of baculovirus infection from unpurified insect homogenates.

2. Methods and materials

2.1. Materials

Monoclonal mouse anti-OpNPV antibodies were purchased from ImmunoPrecise Antibodies (Victoria, BC). Goat-anti-mouse (Fc) (HRP-labelled) secondary antibodies and SuperSignal West Pico chemiluminescent substrate were purchased from Pierce (Rockford, IL). Goat-anti-mouse IgG2b (HRP-labelled) secondary antibodies were purchased from Gentec (Seraing, Belgium). HRP-labelled streptavidin was purchased from Spring Bioscience (Freemont, CA). 5′,3′-Tetramethylbenzidine and diaminobenzidine substrates were purchased from BioFX (Owing Mills, MD). Costar 96-well flat-bottom EIA polystyrene plates were purchased from Bio-Rad Corporation (Mississauga, Ont.). EIA plates were read using a MicroTek plate reader purchased from Bio-Tek Instruments (Winooski, VT). PVDF membranes and autoradiography film (Kodak BioMax MR) were purchased from Amersham (Pitt., PA). Nitrocellulose membrane (AE98 lateral flow membrane) was purchased from Whatmann (Forkham Park, NJ). Rapidograph pen (antibody stripping) was purchased from Koh-i-noor (Leeds, MA). Dry chemicals were purchased from Sigma–Aldrich (Oakville, Ont.).

2.1.1. Laboratory reared Douglas-fir tussock moth larvae

In a previous study (Thorne et al., 2007), second instar DFTM larvae (Goose Lake laboratory strain) were infected by the diet plug method (Kaupp and Ebling, 1990) with 9 OBs/larva or mock-infected with distilled water. Control and treated larvae were arbitrarily selected and individually frozen at −20 °C prior to analyses. Larvae that died prior to selection were collected daily, frozen and later assayed for virus infection.

2.1.2. Field-collected Douglas-fir tussock moth larvae

DFTM larvae were collected by drop cloth from an outbreak site near Cache Creek, British Columbia. Collected larvae were transported to a laboratory at the Pacific Forestry Centre, Victoria, BC, and reared *en masse* (at 25 °C, 50% RH and 16:8 photoperiod) on foliage disinfected with 0.1% sodium