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Environmental Persistence of a Pathogen Used in Microbial Insect Control

Karl M. Polivka, Greg Dwyer, and Constance J. Mehmel[1]

Abstract

We conducted an experimental study of infection, transmission, and persistence of a nucleopolyhedrovirus (NPV) of Douglas-fir tussock moth (*Orgyia pseudotsugata*) to better understand mechanisms determining the efficacy of the virus when it is used as a microbial control agent. In a field experiment, we quantified infection rates of larvae exposed to either Tussock Moth Biocontrol-1, the strain currently used for control by the U.S. Forest Service, or a wild-type strain isolated from a natural population. We first allowed each pathogen to decay on experimental branches for 0, 1, or 3 days before allowing uninfected larvae to feed on the branches, and then we fit both a generalized linear model and an epidemiological model of virus transmission to the infection data. Longer decay of the NPV resulted in lower infection rates, but evidence that overall virus transmission differed between wild and pesticide isolates of NPV was weak. The short persistence time of the virus suggests that it does not last long on foliage, in turn suggesting that application of TM Biocontrol-1 must be carefully timed to ensure maximum mortality.

Keywords: Douglas-fir tussock moth, baculovirus, biocontrol, epidemiological model.

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Introduction

The Douglas-fir tussock moth (*Orgyia pseudotsugata*) (DFTM) is an important pest in western North America, causing extensive damage to Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) forests. As with other forest-defoliator outbreaks (Moreau and Lucarotti 2007), outbreaks of DFTM are usually terminated by epizootics of a highly specific nucleopolyhedrovirus (NPV; Baculoviridae). Because DFTM outbreaks often kill trees, understanding the ecology of the host-pathogen interaction is integral to maintaining overall forest health (Otvos et al. 1987b).

Because of their high degree of species specificity, baculoviruses are often used as environmentally benign insecticides to control DFTM and other forest pests (Moreau and Lucarotti 2007). Full-scale field assays of this and other microbial controls, however, are typically aimed at assessing overall efficacy (Otvos et al. 1987a, 1987b; Shepherd et al. 1984), and so identification of the mechanisms underlying NPV transmission and epizootic dynamics is often a lower priority. Ecological field experiments in contrast make it possible to directly quantify virus transmission rates and persistence times, while allowing for more realistic conditions than dose-response bioassays (Dwyer 1991). Quantification of these key parameters can provide mechanistic details necessary to understand epizootic dynamics in a way that allows managers to make better-informed decisions about NPV application.

Baculovirus infection occurs when larvae consume foliage contaminated by the infectious cadavers of conspecifics (Cory and Myers 2003). Larvae die 8 to 12 days after infection, releasing infectious "occlusion bodies" onto foliage where they are available to be consumed by other larvae, completing the cycle of transmission (Brookes et al. 1978, Dwyer 1991). Occlusion bodies on foliage lose their infectiousness over time, owing to inactivation by exposure to UV radiation and to rains that wash the occlusion bodies off the foliage (Cory and Myers 2003, Raymond et al. 2005). The importance of the breakdown process for DFTM microbial control is poorly understood but could have implications for the timing of spray treatments.

Meanwhile, much of the literature in insect pathology has focused on laboratory dose-response experiments, in which larvae are fed controlled doses, and larvae that do not consume the entire dose are discarded (Cory et al. 2005). Because dose-response experiments do not allow for natural variability in feeding behavior, their results often lead to opposite conclusions from studies carried out in the field. For example, in the gypsy moth-NPV (*Lymantria dispar*) system, larvae-fed virus in combination with white oak (*Overcus alba* L.) foliage in dose-response bioassays have higher infection rates than larvae-fed virus with red oak (*O. rubra* L.) foliage. Infection rates on the two tree species in the field, however, are indistinguishable

because higher feeding rates on red oak counterbalance higher susceptibility on white oak (Dwyer et al. 2005). Similarly, in the tussock moth-NPV system that we study here, susceptibility in bioassays is lower among later instars, but in the field, higher feeding rates lead to higher infection risk in later instars relative to earlier instars (Dwyer 1991).

Field experiments allow for infection under natural conditions, which is an important advantage over laboratory dose-response experiments. This in turn makes it possible to use data from field experiments to quantify transmission and decay parameters in mathematical models, and the resulting model predictions have proven useful for understanding the dynamics of NPV epizootics (Fuller et al. 2012). Such experiments may therefore be useful for understanding NPV dynamics in microbial control.

We therefore carried out a field experiment in which we exposed tussock moth caterpillars to the virus, and used the resulting data to estimate the parameters of a mathematical model of virus transmission and epizootics. This experiment was motivated by two previous studies of spray efficacy during implementation of DFTM control programs (Polivka et al. 2012, Scott and Spiegel 2002). In these studies, application of the virus product Tussock Moth Biocontrol-1 (TMB-1) led to much higher initial infection rates in sprayed plots than in control plots, as expected, yet defoliation rates and final infection rates were effectively the same in the two types of plots. These previous studies suggested that TMB-1 may be less effective than wild-type viruses. Possible explanations for this loss of effectiveness include inadvertent selection for reduced infectiousness in the lab over several rounds of serial passage, and reductions in infectiousness owing to the effects of long-term virus storage (Reed et al. 2003). The large scale of spray programs, however, means that such programs cannot easily be used to test whether infectiousness has in fact changed, while the artificiality of laboratory experiments means that laboratory data often provide unreliable predictions of infection rates in the field (Cory and Myers 2003).

Accordingly, to test whether TMB-1 does indeed have a different phenotype than wild-type virus, we quantified two important phenotypic traits of the two viruses in a field experiment. Mathematical models of epidemics have shown that two key parameters determining infection rates are, first, the rate of horizontal transmission of the virus, and second, the rate at which the virus breaks down on foliage (Dwyer et al. 1997). We therefore designed our experiment so that we could estimate both the transmission and the decay rate for each NPV isolate by fitting a

mathematical transmission model to the data (Fuller et al. 2012). Here we demonstrate differences in mortality rate between wild and biocontrol isolates of NPV and show that they are not explained by differences in transmission or decay rates. We show that TMB-1 is just as infectious as one wild-type virus isolate and illustrate the usefulness of mathematical models for interpreting data on microbial control agents.

Methods

To produce insects for our experiments, we mated feral insects collected from the Methow Ranger District, Okanogan-Wenatchee National Forest, Washington. We reared offspring from these matings to the third instar in an incubator following standard protocols (Chauthani and Claussen 1968). We used third instars because infection rates in virus epizootics in nature typically increase sharply when larvae reach the third instar (Dwyer 1991, Otvos et al. 1987a). By initially rearing insects in the lab, we ensured they did not become infected before the experiment began.

We conducted experiments in a mixed Douglas-fir/ponderosa pine (*Pinus ponderosa* Lawsone C. Lawson) stand at a site on Horse Lake Mountain, Chelan County, Washington, within the range of the tussock moth, but tussock moth densities were low enough that the virus was undetectable on our experimental trees. We randomly selected and assigned treatments to single branches (area ~ 1 m^2) on individual trees, such that each branch was a separate replicate. We obtained Tussock Moth Biocontrol-1 (TMB-1) from the stock (Lot #4) used by the U.S. Department of Agriculture Forest Service (Corvallis, Oregon) in spray programs, and we isolated a wild-type NPV strain from cadavers collected during a natural epizootic on the Methow Ranger District.

Note that TMB-1 was isolated from a wild-type strain from Goose Lake, Oregon, decades before our study[2] (Martignoni 1999), whereas, the wild-type isolate was collected in an outbreak in 2010 in an area that had been sprayed with TMB-1 during a previous outbreak in 2001. Because sprayed baculovirus typically has a very short half-life (Fuller et al. 2012), the 9 years that elapsed from when TMB-1 was applied and the wild-type isolate was collected means that it is highly unlikely that the two are the same, or even closely related. Moreover, as we will demonstrate, our results show clearly that the phenotypes of the two strains are significantly different.

Each experimental branch was sprayed with 4.5 mL of a solution of deionized water containing 1,000 occlusion bodies of NPV per µL, while control branches

[2] **Magelssen, R. 2010.** Personal communication. Entomologist, Forest Health Protection, Okanogan-Wenatchee National Forest, 1133 N Western Ave., Wenatchee, WA 98801.

were sprayed with deionized water only. We used this concentration of NPV because in an earlier dose-response bioassay in the laboratory, doses this high and above consistently caused at least 95 percent mortality. We carefully measured the concentration of the virus solution to ensure that it was the same for the two virus isolates, and we treated each experimental branch with a single isolate. To generate decay treatments, we applied virus to the branches 3, 1, or 0 days before adding healthy larvae. Six branches were assigned to each virus and decay treatment (total $n = 36$), alongside six control branches. There were no infections on control branches, indicating that there was no detectable virus on the branches before our experiments began, so we do not discuss controls further.

After adding 15 larvae to each branch, we enclosed branches in mesh bags that prevented larval escape and resisted further virus decay (Fuller et al. 2012). After 7 days, a period short enough to ensure that none of the initially uninfected larvae died from infection, which could have caused further transmission, we removed each branch to the lab and reared all larvae in individual cups of artificial diet in an incubator until death or pupation. We confirmed virus deaths by autopsy and recorded the number of infected individuals on each branch.

We analyzed our data by carrying out a test of statistical significance, and by fitting transmission models to the data. To carry out a significance test, we fit a generalized linear model (GLM) to our data, assuming a binomial error distribution, and we asked whether virus decay and/or virus strain affected overall infection. In the GLM, we included decay time, isolate (TMB-1 or wild) and an interaction term (decay · isolate). We tested the fit of model deviance values to the data with a chi-square test and by examining plots of residuals.

In field decay experiments such as ours, it is possible to simplify a standard epidemiological model (Keeling and Rohani 2007), and then to fit the model to data (Fuller et al. 2012). This approach produces an expression for the fraction infected at the end of the experiment: $S(t)/S(0) = \exp(-\nu P(0) \exp(-\mu T))$, where $S(t)/S(0)$ is the fraction uninfected at the end of the experiment (time = t), and T is the amount of time for which the virus was allowed to decay. The parameters ν, the transmission rate, and μ, the decay rate, are then estimated using maximum likelihood and a nonlinear fitting routine, assuming a binomial likelihood function. These parameters in turn allow calculation of the disease-density threshold, the lowest host density at which the introduction of a small amount of virus will lead to at least a few new infections (Lloyd-Smith et al. 2005), which is equal to μ/ν (Dwyer et al. 2000).

We then used Akaike information criterion (AIC) analysis to choose between models that made different assumptions about the effects of differences in virus isolate on virus transmission and decay. Specifically, the models assumed (1) no

decay ($\mu = 0$) in either wild-type NPV or TMB-1 with either the same or different v (two models), (2) each isolate has a unique v and μ, (3) each isolate has a unique μ but the same v, (4) unique v but same μ, or (5) same v and μ, for a total of six models. To choose the best of the six models, we used the AIC model-selection criterion with adjustments that allowed for small sample size and for the possibility of extrabinomial variation ("overdisperson"), yielding quasi-AIC $(QAIC)_c$ scores (Burnham and Anderson 2002). For our purposes, an important feature of AIC analysis is that it explicitly allows for parsimony. In particular, if a simpler model is within 2 $QAIC_c$ points of a more complex model, parsimony dictates that the simpler model be considered the best description of the data. Because the models that we compared include mechanistic descriptions of transmission, AIC analysis thus allowed us to test whether the differences revealed by our test of statistical significance have any biological significance.

Results

Increasing decay time strongly lowered the infectivity of both isolates (fig. 1). The wild-type isolate was slightly more infectious across decay treatments as compared to Tussock Moth Biocontrol-1. The GLM analysis showed that both the decay treatment and the isolate treatment were strongly statistically significant (decay time effect: Deviance = 52.99, df = 32, $P < 0.0001$; virus isolate effect: Deviance = 10.24, df = 34, $P = 0.001$). Decay rates, however, did not significantly differ between virus strains (Deviance = 0.258, df = 30, $P = 0.314$). A plot of residual vs. fitted values indicated no systematic lack of fit of the GLM to the data. We therefore conclude that TMB-1 is phenotypically distinct from the wild-type strain.

Results for the epidemiological model-fitting were qualitatively similar in that the best-fit model assumes that the two virus isolates differ in transmission rate, ν, but not decay rate, μ (table 1). However, the model that assumed **no** differences between the two isolates had a $\Delta QAIC_c$ score within 2 points of the score for the best model (table 1). We therefore conclude that, although there was a statistically significant difference in the infection rates between the two isolates in our experiment, this difference is not biologically meaningful.

Our estimates of the disease-density thresholds for each strain provide further support for the conclusion that the infectiousness of TMB-1 is not meaningfully different than the infectiousness of the wild-type isolate. The transmission rates of the two virus strains, as estimated by fitting the best model, had respective medians of $0.103/day/m^2$ (TMB-1; bootstrapped 95 percent CI: 0.049 to 0.193) and $0.144/day/m^2$ (wild-type; 95 percent CI: 0.076 to 0.272). Because the best model indicated no difference in decay rate, μ, we estimated a single median decay rate

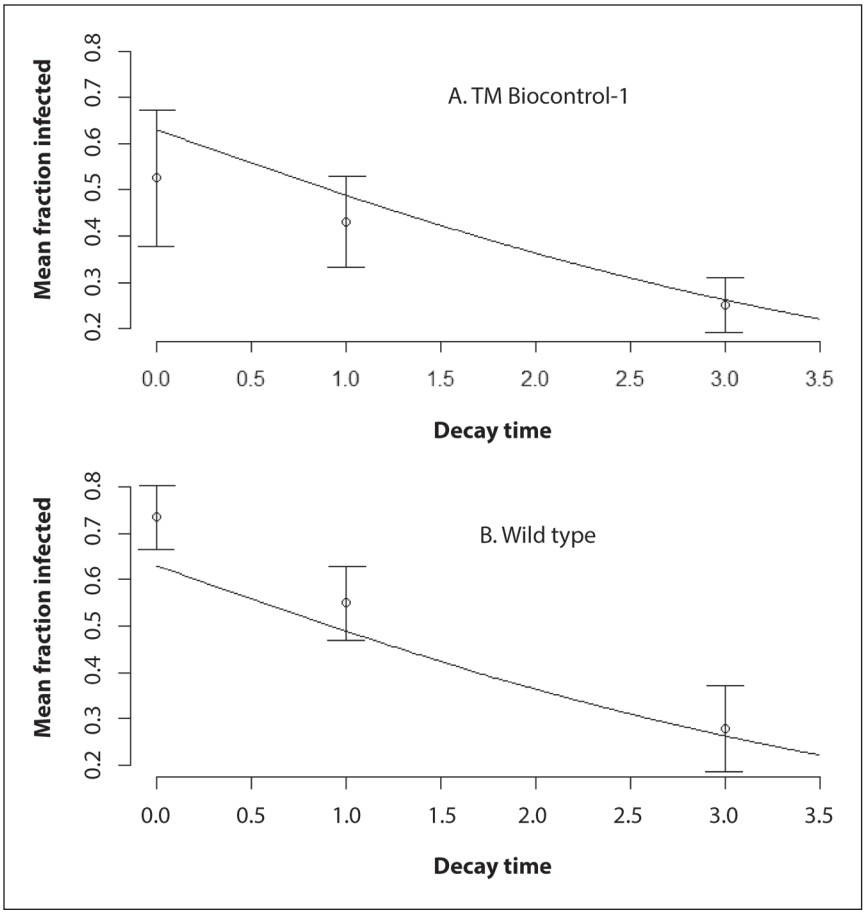

Figure 1—Fraction infected versus decay time for (A) Tussock Moth Biocontrol-1 and (B) a wild-type isolate. Points show the data and error bars are one standard error of the mean. Lines show the best-fit version of a transmission model that assumes different transmission rates but identical decay rates for the two isolates (the best model in table 1).

Table 1—Quasi-Akaike information criteria $_c$ ($QAIC_c$) scores and negative log-likelihood (-LL) for models that make different assumptions about the effects of virus strain on virus transmission (ν) and decay (μ)[a]

Model assumptions	Parameters	-LL	$\Delta QAIC_c$
Identical ν's, μ = 0	2	150.0	17.42
Different ν's, μ = 0	3	142.9	17.32
Identical ν's, identical μ's	3	102.3	0.70
Identical ν's, different μ's	4	101.7	3.59
Different ν's, identical μ's	**4**	**92.9**	**0.00**
Different ν's, different μ's	5	90.6	2.68

Note: Some branches had more than 50 percent nondisease mortality, but leaving out such mortality from our analyses did not affect our conclusions.

[a] The best model is in bold face (variance inflation factor = 2.5, indicating only modest heterogeneity in variance).

of 0.357/day (bootstrapped 95 percent confidence intervals, CI: 0.656 to 0.058), giving an average persistence time of 2.80 days. We then have 95 percent CIs on the disease-density threshold of 1.34 to 6.45 larvae/m^2 for TMB-1 and 0.661 to 4.05 larvae/m^2 for wild type, showing that the difference in disease-density threshold between the two isolates is very slight. These values themselves, however, compare well with observations of at least some baculovirus infections in natural DFTM populations at minimum densities of roughly 1 to 10 larvae/m^2 (Otvos et al. 1987a).

Discussion

Our finding that the wild-type strain produced a significantly higher infection mortality than Tussock Moth Biocontrol-1 demonstrates that the two strains are indeed phenotypically different, despite approximately equivalent transmission and decay rates. The data thus reject the hypothesis that the two strains are the same, or closely related. As we described earlier, this is not surprising, given the short persistence time of NPVs, as calculated here and in Fuller et al. (2012). It is therefore unlikely that virus sprayed on the foliage would persist long enough to give rise to the wild isolate collected 9 years later. Secondly, hosts generally impose intense selection on pathogens and the pathogen's short generation time within a host facilitates the rapid accumulation of variation on which selection may act (Cory et al. 2005, Keeling and Rohani 2007). Third, selection imposed by hosts can cause NPV to accumulate substantial base-pair differences detectable within sites and among sites as close as 2 km apart (Williams et al. 2011), which would similarly lead to rapid divergence. Therefore, after 9 years of divergence time, it appears that the wild-type strain is, at most, a distant relative of TMB-1.

The AIC analysis nevertheless revealed that the phenotypic differences in infection mortality between the two virus isolates were unlikely to be of much biological significance in terms of the rate at which NPV can spread through a population, either naturally or via biocontrol efforts. We therefore conclude that differences between TMB-1 and wild strains are likely to be of little concern in management programs. This is important because, as we mentioned earlier, in previous studies, forest stands treated with TMB-1 had similar defoliation rates and final infection rates to untreated stands, even though initial infection rates in the untreated stands were considerably lower (Polivka et al. 2012, Scott and Spiegel 2002). These observations led to the concerns that (1) long-term storage has reduced the efficacy of TMB-1, or (2) wild strains are inherently more virulent than TMB-1. Our results therefore suggest that concerns about long-term storage of TMB-1 are unfounded, because transmission rates are very similar and decay rates are identical for the

TMB-1 and the wild-type isolate. We further suspect that inadvertent selection in the lab for reduced efficacy is also a nonissue, with the proviso that we tested only one wild isolate, so this latter conclusion is preliminary, pending further experiments with additional wild isolates.

We do not have a mechanistic explanation for the GLM result showing higher mortality in the wild strain, but our results suggest that the observations in Polivka et al. (2012) and Scott and Spiegel (2002) were **not** due to strong differences in decay rates or transmission rates between TMB-1 and wild-type virus. In future work, we will therefore test the alternative explanation that small initial inputs of virus in control plots may lead to overall infection rates that match the rates in treated plots. In particular, rising infection risk and occlusion body production among later instars (Dwyer 1991) may mean that small initial inputs of virus can lead to very high infection rates among late instars. We will also test this hypothesis by fitting mathematical models to a combination of the data that we report here, and the data in the previous spray studies. More immediately, however, we conclude that the overall infectiousness of TMB-1 closely approximates that of naturally occurring NPV.

A second interesting feature of our results is that the relatively short persistence time of the virus (<3 days) is similar to that estimated by Fuller et al. (2012) for the gypsy moth NPV. When compared with the development time of DFTM larvae (~ 10 weeks), this estimate suggests that it is unlikely that the virus would persist until later instars that have higher infection risk are present in the population. This is important because previous experiments with this (Dwyer 1991) and other (D'Amico et al. 1998, Dwyer et al. 2005) insect-NPV systems have provided overwhelming evidence that later instars are at much greater risk of infection than earlier instars. This effect presumably occurs because the higher feeding rate of later instars leads to a high exposure risk, despite the higher resistance to infection observed in later instars (Dwyer 1991).

Moreover, infection rates in treated plots are often quite high late in the season, long after the initial application of the virus (Otvos et al. 1987a, Polivka et al. 2012). The short persistence times that we observed thus support the suggestion of Otvos et al. (1987a) that high late-season infection rates, whether in sprayed populations or in populations with natural epizootics, are due to successive rounds of transmission rather than to the long-term persistence of the applied virus. It therefore seems likely that secondary transmission plays a key role in microbial control of populations, but such transmission is only rarely accounted for in management practice. In particular, current practice is to spray the virus on second instars, which feed

slowly and therefore have relatively lower infection risk (Dwyer 1991). It may instead be better to spray later instars, using lower amounts of virus, even at the cost of reducing the number of virus infection cycles that can be completed before the insects pupate.

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English Equivalents

When you know:	Multiply by:	To find:
Kilometers (km)	0.621	Miles
Square meters (m^2)	10.76	Square feet
Milliliters (mL)	.0338	Ounces (fluid)
Microliters (µL)	3.381	Ounces (fluid)

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