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Detection of Low Levels of Baculovirus for Outbreak-Terminating Epizootics in Defoliating Insects

Spencer Carran, Carlos M. Polivka, Joseph R. Mihaljevic, and Greg Dwyer[1]

Abstract

Recent empirical and theoretical studies have indicated that epizootics of baculoviruses in defoliating insects may result in the termination of outbreaks starting from lower initial infection rates than previously believed. This suggests that natural epizootics can preempt the need for costly and labor-intensive pest management measures because natural epizootics reduce or end outbreaks before substantial defoliation occurs. Such cases, however, require the ability to detect small amounts of infection at the beginning of the population's life cycle. At hatching, Douglas-fir tussock moth (*Orgyia pseudotsugata*) larvae become infected by baculoviruses on the surface of their eggs. Prior to the larval season in an outbreak area, egg masses are collected and assayed for the presence of viruses; however, a large number of eggs may need to be sampled to detect low infection rates in which biocontrol measures are not needed. Here we used simulated sampling to detect infection rates ranging from 10^{-3} to 10^{-4} and considered whether multiple potential probability distributions of the actual infection rate affected detection. We showed that the level of sampling is considerably higher than in previously published protocols, but that increased effort via either more egg masses sampled (with a fixed number of eggs per egg mass), or more eggs per egg mass sampled without collecting more egg masses, equally improved the accuracy of detecting the virus.

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

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Introduction

Native insects are important to forest managers for many reasons, including their impacts on economic, ecologic, and recreational resources (Otvos et al. 1998). The Douglas-fir tussock moth (*Orgyia pseudotsugata*) (DFTM) is a native pest in western North America. In episodic outbreaks, its larvae cause extensive defoliation to Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and true fir (*Abies* spp.) forests. Populations of DFTM are strongly cyclical and are nearly undetectable in the years between outbreaks. Populations likely persist in the crowns of trees, where life stage sampling is often impractical, and defoliation goes unnoticed because levels are too low to be reported in aerial surveys. When populations reach outbreak levels, severe defoliation can lead to tree mortality; however, surviving trees can be vulnerable to secondary infestation by bark beetles or other insect pests. Tree mortality can exacerbate the effects of climate change (Slaney et al. 2009) or fire and may result in economic losses via damaged timber or by reducing the quality of recreation areas. Outbreak DFTM populations typically persist for 2 to 4 years before being terminated by epizootics of a species-specific baculovirus combined with parasitoids and predators. Baculoviruses can be used as insecticides to reduce defoliation damage without affecting non-target species (Moreau and Lucarotti 2007). The baculovirus that is used to control DFTM is known as TM Biocontrol-1 (TMB-1); it has been used to neutralize outbreaks across the range of the insect.

High-density DFTM populations often collapse because of the native baculovirus, even in the absence of control measures because the transmission of the baculovirus is strongly density dependent (Mihaljevic et al., n.d.; Polivka et al. 2012). This suggests that surveys of DFTM densities could allow entomologists to identify populations that are large enough to likely experience an outbreak-terminating epizootic (Scott and Spiegel 2002). Management decisions to treat forest stands with

TMB-1 are based on the rationale that infecting a higher fraction of the population than the natural infection rate will accelerate the epizootic (Shepherd et al. 1984). By artificially causing an epizootic to spread faster, biocontrol may reduce defoliation to a level below what would occur in a natural epizootic (Sheehan et al. 2004). Linked with the decision to apply TMB-1 are laboratory assays to estimate an initial infection rate with the recommendation that spraying is not necessary when laboratory infection rates are 25 percent or higher (Stelzer 1979). Mathematical models derived from epidemiological theory (susceptible-exposed-infectious-recovered [SEIR] models) (Keeling and Rohani 2011), can be adapted to insect-pathogen systems to describe the spread of a nucleopolyhedrovirus (NPV) (e.g., Dwyer et al. 1997, 2000; Mihaljevic et al., n.d.). Such models, developed for DFTM populations, show that small initial infection rates can lead to very high final infection rates (Mihaljevic et al., n.d.). The specific model is:

$$\frac{dS}{dt} = -\overline{\nu}e^{ct}\, SP\left[\frac{S(t)}{S(0)}\right]^{C^2},$$

$$\frac{dE_1}{dt} = -\overline{\nu}e^{ct}\, SP\left[\frac{S(t)}{S(0)}\right]^{C^2} = m\delta E_1,$$

$$\frac{dE_i}{dt} = m\delta E_{i\text{-}1} - \delta E_i (i = 2,\ldots,m),$$

$$\frac{dP}{dt} = m\delta E_m - \mu P.$$

This model produces disease dynamics showing the course of the epizootic that terminates, or nearly terminates, an outbreak across a range of initial population sizes (S), even for extremely low infection rates. The model incorporates the density-dependent transmission rate, ν, which can be estimated from natural epizootics, transmission experiments, and biocontrol projects. The model also assumes that transmission varies among individuals (C) and that individuals transition through a set of pathogen exposure classes (E_i). Once statistically robust estimates have been obtained from fitting the model to the various types of data listed above, the model can be used as a tool to predict the timing and intensity of epizootics across a range of population sizes and initial infection rates (fig. 1) (Mihaljevic et al., n.d.).

A key result from the fitting of this model is that the disease density threshold, i.e., the minimum infection rate required to cause an epizootic, is quite low, particularly relative to the 25 percent infection rate upon which biocontrol decisions are based (Stelzer 1979). This low disease density threshold suggests that laboratory assays should be designed to detect infection rates as low as 10^{-3} to 10^{-4}. This may require the testing of far more than the recommended 50 eggs per egg mass indicated in Stelzer (1979), which is very time and labor intensive but, given the costs associated with TMB-1 or other treatment applications, may still be more economical. It may be possible to streamline such laboratory efforts by having a more

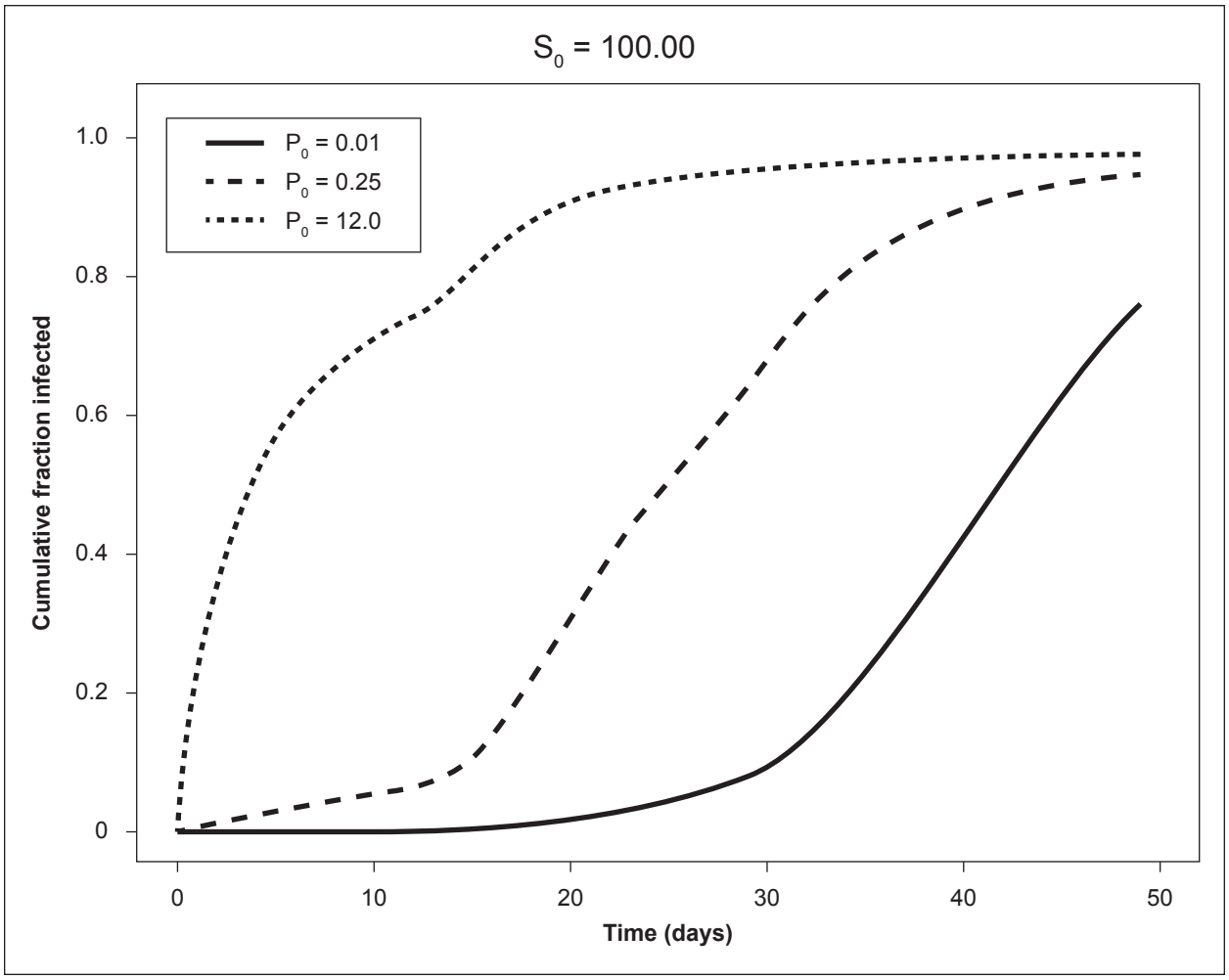

Figure 1—Cumulative fraction infected (FI) over time (starting from hatching) in a hypothetical population with starting density S_0 = 100. Three initial virus densities (expressed in infectious cadaver equivalents/m^2, P_0) are compared, resulting in varying intensity of epizootics, always resulting in the population crashing.

accurate estimate of the amount of sampling (in terms of the number of egg masses or eggs) required to detect such low infection rates.

Here we describe simulations that generate virus detection probabilities for different levels of sampling effort in the virus assays that occur, as described, prior to decisions to apply biocontrol measures.

Methods

We developed a simulation model to determine the probability that a given level of sampling effort will identify the presence of the virus at some true infection rate within a site. Infection rates may not be constant across all egg masses within a site, so we drew hypothetical values from a beta distribution to use as the infection rate of a given egg mass. The beta distribution is defined by two positive shape parameters, α and β, and is bounded on the interval [0, 1] with mean $\frac{\alpha}{\alpha+\beta}$. To consider

whether the distribution of infection rates across egg masses is overdispersed, we considered multiple parameterizations of the beta distribution, scaled to retain the same mean but with different variances. We then made a number of binomial draws within each egg mass based on the number of eggs sampled and the egg mass infection rate drawn from the beta distribution. After simulating 10^6 realizations of this sampling process, we determined a detection probability equal to the fraction of simulations in which we expect to find at least one infected insect. Assuming that each egg mass can produce up to 300 eggs and that up to 30 egg masses may be collected at a site, we then determine detection probabilities for each combination of the number of egg masses and number of eggs sampled per egg mass. It may not always be possible in practice to collect 30 egg masses at a given site, particularly in the early years of an outbreak, but we consider it an upper bound on a practicable sampling effort to detect the virus.

Results

Our simulations indicated that it is possible to detect the low initial infection rates sufficient to cause an epizootic with reasonably high probability. However, the level of sampling effort can become impractical when the true infection rate is very low. The difficulty in detecting extremely low infection rates is due in part to the field and laboratory time involved; however, it is also because even the maximal sampling effort results in a moderate probability of failing to detect the virus even where it is present.

If the true virus infection rate is 1/1,000, then even sampling 50 eggs per egg mass requires a relatively large number of egg masses sampled to have a high probability of detecting the virus. Under that design, sampling only 16 egg masses yields a 50 percent chance of detecting the virus, and surveying 30 egg masses would still leave a one-third chance of failing to detect any virus (fig. 2). Alternately, it is possible to obtain high detection probabilities by sampling a greater number of eggs per egg mass; a sampling effort of 150 eggs, each hatched from 15 egg masses, would yield a detection probability of more than 90 percent.

Detecting lower levels of the virus requires significantly more effort. If the true infection rate is 1/10,000, then even a comprehensive survey examining 300 eggs, each in 30 egg masses (for a total of 9,000 eggs), yields a 40 percent chance of failing to find any positive virus samples (fig. 2). Note that the lines of equal detection probability indicated in figures 2 and 3 are also lines of equal effort; so increasing the total number of eggs sampled yields greater detection probabilities, whether they were obtained by collecting more egg masses or by hatching a greater number of eggs per egg mass. Under simulation conditions that consider the overdispersion of virus distribution among egg masses, detection probabilities were not substantially affected (see app.).

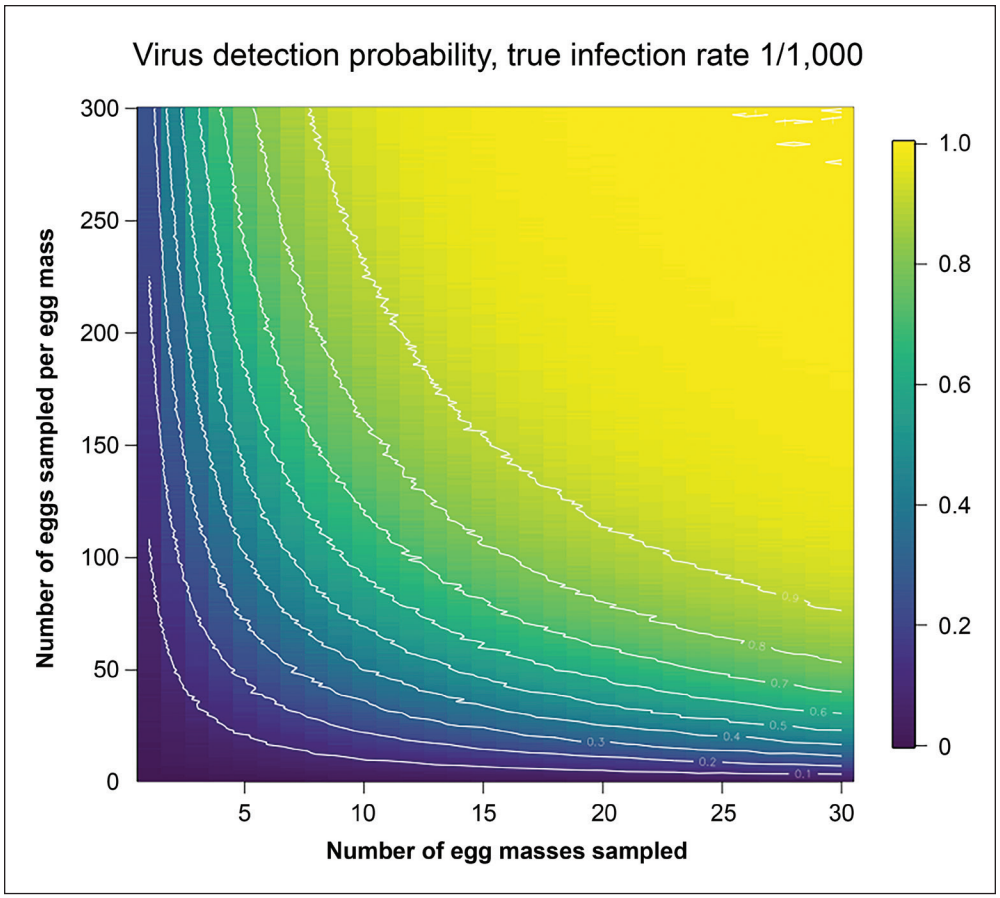

Figure 2—Simulation results that show the probability of detecting virus with a given level of sampling effort, with colors towards yellow indicating a higher probability of finding the virus (scale on right). The white lines (placed at 10 percent detection probability intervals) indicate tradeoffs between the number of egg masses sampled and number of eggs per egg mass that yield the same detection probability. If the true initial infection rate is 10^{-3}, relatively modest sampling effort is likely to detect at least some virus presence.

Discussion

From both theory (Mihaljevic et al., n.d.) and observations (Polivka et al. 2012), outbreak-terminating epizootics can result from low initial infection rates. Because epidemiological models can also predict the time course of epizootics (fig. 1), managers may wish to reconsider the decision to treat outbreaks that may end more rapidly as a result of natural NPV infection rather than the available treatment options (TMB-1, *Bacillus thuringiensis*). Based on predictions described in Mihaljevic et al. (n.d.), determination of the likelihood of population crashes may require the ability to accurately detect virus infection rates that are much lower than the threshold used for biocontrol management decisions (Stelzer 1979). Natural epizootics that have occurred in untreated reference stands during biocontrol programs (e.g.,

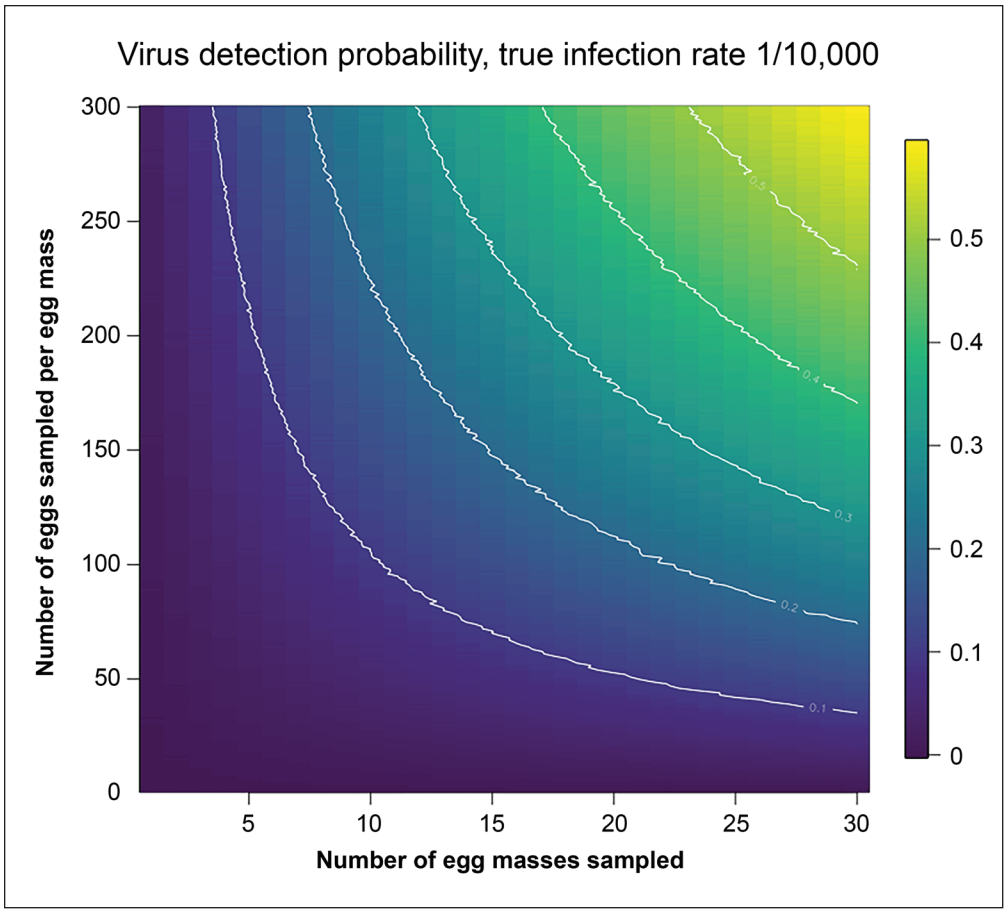

Figure 3—If the true initial virus infection rate of Douglas-fir tussock moth eggs is 10^{-4}, even extensive sampling of egg masses may fail to detect the presence of virus.

Polivka et al. 2012, Scott and Spiegel 2002,) appear to have originated from low rates, leading to the estimation of a low disease density threshold (Mihaljevic et al., n.d.). Our simulations show that relatively high sampling effort is required to detect virus infection rates consistent with the low estimated disease density threshold in Mihaljevic et al. (n.d.). They assign specific detection probabilities to the tradeoff between collecting more egg masses or conducting virus assays on more eggs per egg mass. The primary driver of virus detection probability is the total number of eggs sampled, regardless of whether additional sampling effort is devoted to collecting more egg masses or to examining a higher number of eggs within each egg mass. Therefore, the choice about the organization of sampling effort should be driven primarily by practical tradeoffs between additional field sampling to collect more egg masses and the laboratory capacity necessary to assay the eggs within each egg mass.

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Appendix

We considered multiple possible distributions of infection rates across egg masses to consider whether our detection probabilities may be biased by overdispersion. As can be seen in figures 4 to 6, counterintuitively, varying distributions of infection rates across egg masses did not discernibly affect detection probability or the relative benefit of sampling either more egg masses or more eggs per egg mass. As long as there was some non-zero rate of infection in each egg mass, our simulation found similar benefit to sampling either more egg masses or more eggs. The only scenario in which we were able to identify a potential impact of overdispersion on viral detection probabilities was the extreme and unrealistic hypothetical depicted in fig. 7, in which each egg mass has an infection rate of 0 or 1 (i.e., consists of only uninfected eggs or only infected eggs). In this case, no additional information is gained by sampling more than one egg from a given egg mass, and detecting the virus requires collecting many more egg masses than would be practical.

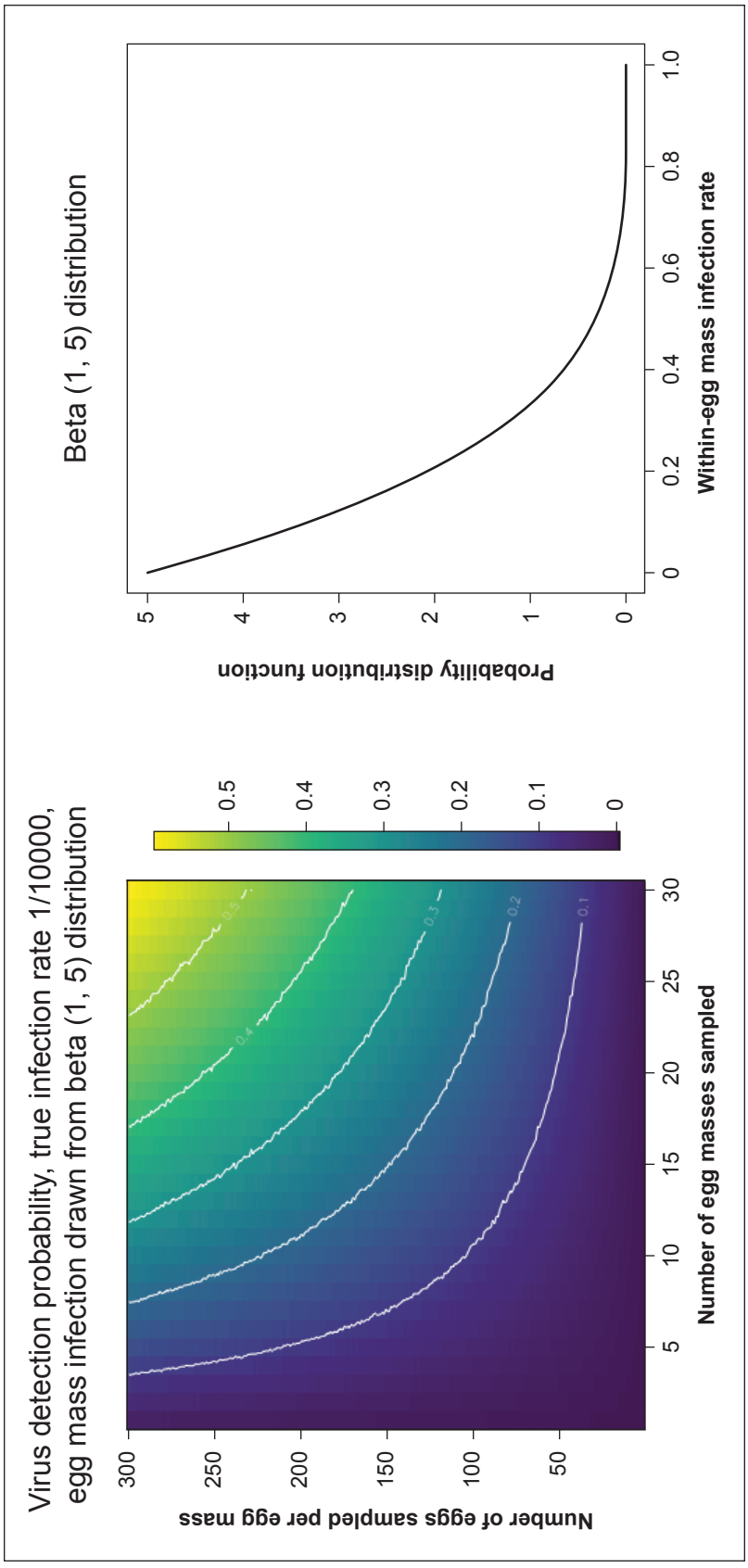

Figure 4—Detection probabilities as a function of effort with a beta(1, 5) distribution of egg mass infection rates, corresponding to a variance of approximately 0.02 in the distribution of infection rates. The right panel shows the corresponding beta distribution, in which most egg masses have low infection rates, and a smaller number of egg masses have high infection rates.

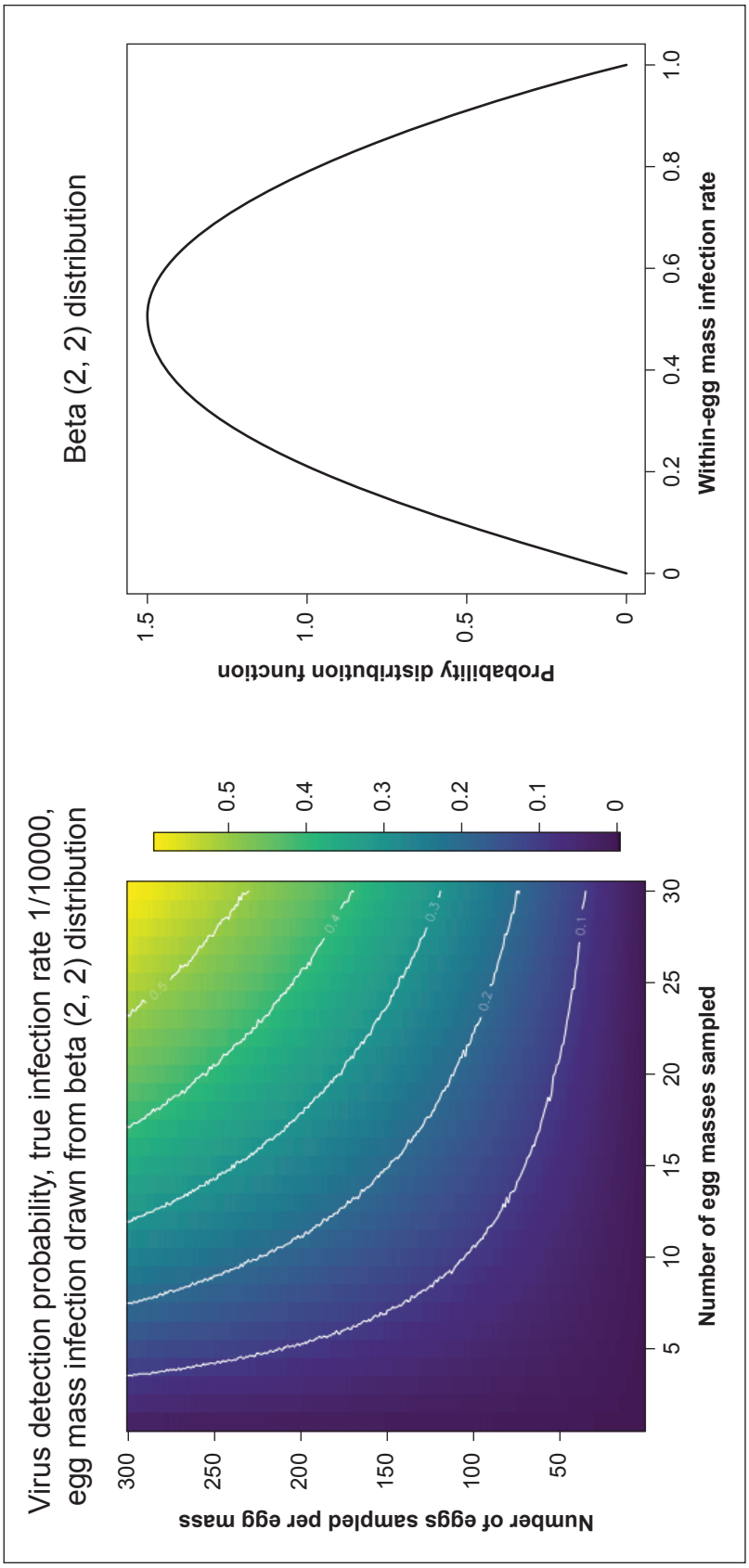

Figure 5—Detection probabilities as a function of effort with a beta(2, 2) distribution of egg mass infection rates, corresponding to a variance of 0.05. In this example, most egg masses have an average fraction of infected eggs, with few egg masses having either very high or very low infection rates.

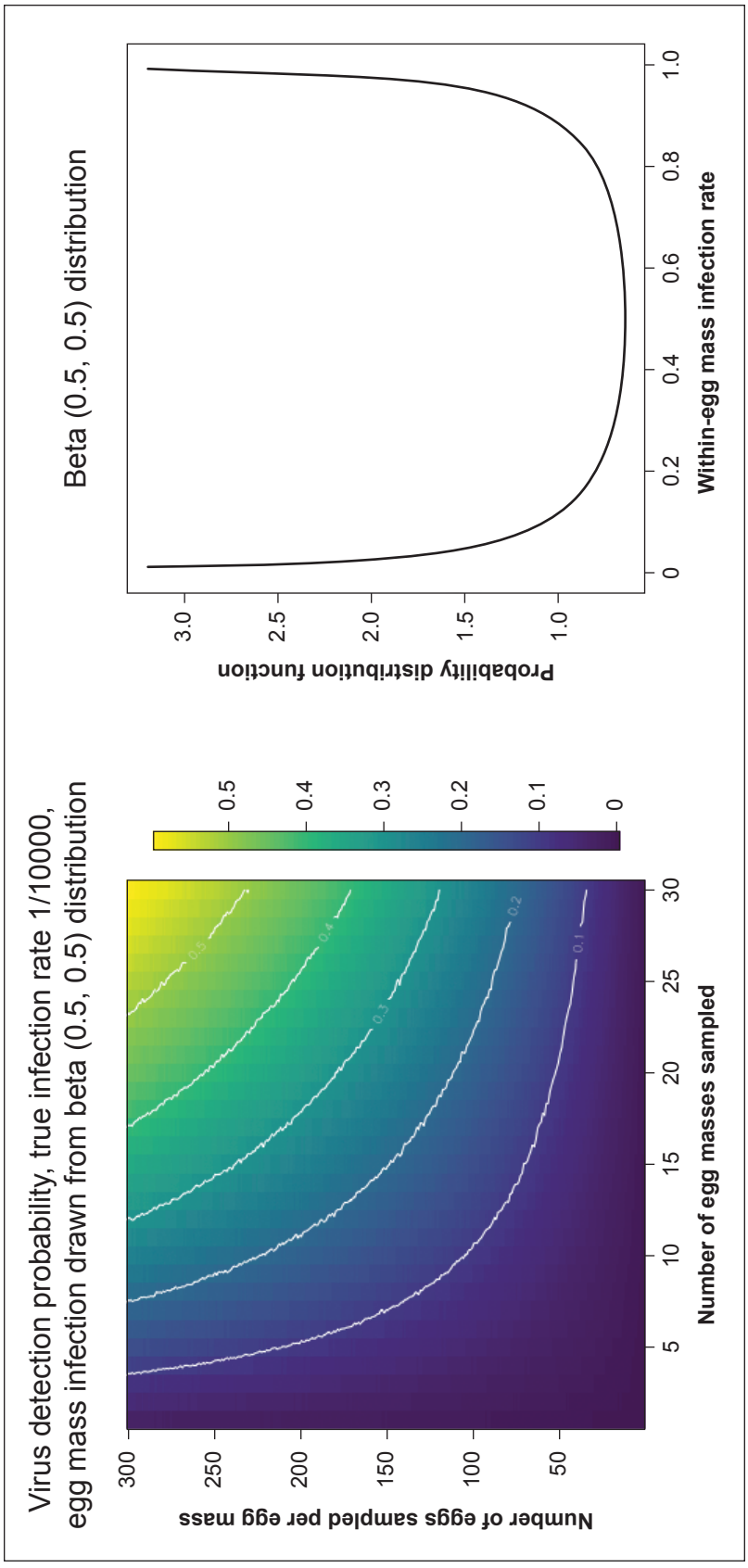

Figure 6—Detection probabilities as a function of effort with a beta(0.5, 0.5) distribution of egg mass infection rates, corresponding to a variance of 0.125. In this example, infection rates are likely to be either very high or very low, with very few egg masses showing intermediate rates of infection.

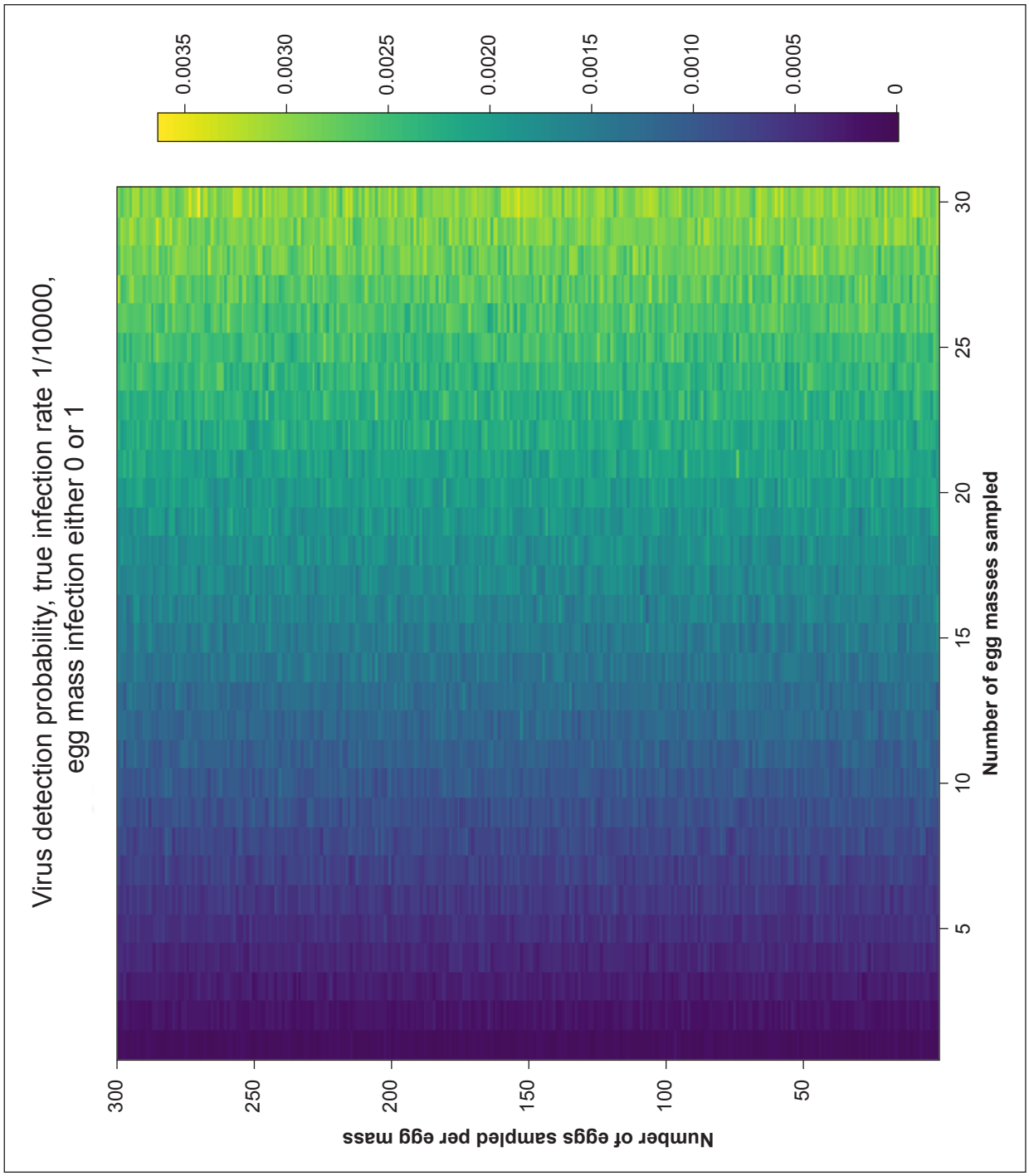

Figure 7—In the most extreme case of overdispersion, in which all egg masses have infection rates of either 0 or 1, detection probabilities are a function only of the number of egg masses sampled.

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