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Bacillus thuringiensis impacts on primary and secondary baculovirus transmission dynamics in Lepidoptera

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ABSTRACT

Synergistic interactions between entomopathogenic micro-organisms can potentially be exploited to improve biological control of invertebrate pests but empirical data at the population level describing multiple-pathogen transmission dynamics is lacking. We examined how co-inoculation of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) and the baculovirus *Panolis flammea* nucleopolyhedrovirus (*Pafl*NPV) in an experimental field population of Lepidopteran *Mamestra brassicae* larvae impacted on viral transmission dynamics. We determined how the presence of *Btk* influenced primary and secondary *Pafl*NPV transmission. When *Btk* was co-inoculated with *Pafl*NPV, there was increased proportional viral mortality in primary transmission studies compared to plots with virus alone. A delay of up to 4 days between applications of *Btk* and *Pafl*NPV did not impact on primary viral mortality, indicating that a lag between inoculations was unlikely to affect the biocontrol potential of the two pathogens. Viral yields from cadavers in plots with *Btk* present were significantly lower than those from plots with virus only, and secondary cycling to introduced secondary transmission larvae was significantly reduced. Baculovirus transmission (in terms of the proportion of uninfected larvae in different treatments) was described by a 'refuge' model that allowed for heterogeneity in susceptibility and pathogen exposure. We discuss how transmission may be potentially affected by factors such as host feeding rate, spatial distribution of virus and interactions between pathogens within the insect host. This study improves understanding of the impact of pathogens within host populations and how mixtures of pathogens may be exploited for biocontrol of insect pests.

1. Introduction

Naturally occurring microparasitic organisms (viruses, bacteria, fungi and protozoa) are important regulators of invertebrate pests in agronomic ecosystems (Smith et al., 2011) and can have significant impacts on the population dynamics of their host insects. Entomopathogenic micro-organisms have been successfully developed for biological control of agricultural, horticultural, forestry, turf/lawn pests and stored product pests (Bailey et al., 2010; Lacey et al., 2015; Sanahuja et al., 2011). They represent an environmentally benign alternative to chemical pesticides and their benefits include: safety for non-target/beneficial organisms as many are specific to certain invertebrate species or groups (although some may attack several different species); reduction of pesticide residues on crops; management of pesticide and *Bacillus thuringiensis* (*Bt*) resistant populations; and provide long-term control by secondary and longer cycling in host insect populations. One of the key current challenges to the expansion of biopesticides into the predominately synthetic chemical market for invertebrate pest control is to integrate chemical and biological control in a reliable and predictable manner as complimentary components within integrated pest management systems (Lacey et al., 2015). However, biopesticides are also sometimes perceived as being inconsistent in their performance in pest management for reasons such as lack of broad spectrum activity, slower speed of kill and differential activity under varying abiotic conditions. One way to overcome these limitations may be combined use of entomopathogenic micro-organisms with both conventional synthetic pesticides and other applied micro-organisms/pathogens to synergise efficacy and reduce variability of control.

Baculoviruses are a group of double-stranded DNA invertebrate pathogenic viruses that have been used extensively for biological control. They predominantly infect Lepidoptera and can cause epizootics within natural populations of insects (e.g. Elkington, 1990; Myers and Cory, 2013), as they are able to persist in the environment and so can cause outbreaks via multiple cycles of infection (Reilly and Elderd, 2014; Murdoch et al., 1985). Baculoviral

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material is occluded in either polyhedrin or granulin protein matrixes forming an occlusion body, a morphological feature used to define the genera respectively as the nucleopolyhedroviruses (NPVs) or the granuloviruses (GVs) (King et al., 2009). Infectious occlusion bodies can be applied as formulated products containing large numbers of infectious particles, akin to those of conventional chemical applications. This strategy is often referred to as inundative, when the biological agent is introduced in sufficient quantity to achieve control without significant reproduction of the microbial agent. This has led to much success in the development of baculoviruses as biopesticides for application in field environments.

Due to their ease of study, experimental Lepidoptera–baculovirus systems have been used extensively to evaluate pathogen transmission dynamics as virus–host systems can be manipulated in a controlled way that can inform pathogen–host interactions and disease models (e.g.; Elderd, 2013; D'amico et al., 1996; Dwyer, 1991, 1994; Goulson et al., 1995; Hails et al., 2002; Reeson et al., 2000). Key parameters such as transmission of infective material, pathogen persistence in the environment and yield of infectious particles is known to contribute significantly to pathogen population dynamics (Anderson and May, 1981). However, empirical data at the population level is generally sparse (Tompkins et al., 2010) although there are good examples from baculovirus control of Lepidoptera in forestry and fruit (e.g. Otvos et al., 1987; Lacey et al., 2008). These data are essential to evaluate the success of biological control agents in suppression of pest populations through understanding how pathogens can persist and transmit within host populations. Data-parameterised models that have been well tested under field conditions could then be used to inform how management strategies may affect the long-term dynamics of a system.

The bacterium *Bacillus thuringiensis* Berliner (*Bt*) is the most widely used biological control agent in the world (Sanahuja et al., 2011). *B. thuringiensis* is recognised as a complex of rod-shaped, gram positive bacteria, which under optimal conditions sporulate to form crystalline parasporal bodies containing proteinaceous insecticidal delta-endotoxins. Once bacteria are ingested by an insect there is very quick paralysis of the gut and cessation of feeding. Infection with sublethal doses of *Bt* has a number of effects on Lepidoptera larvae such as; reduction in larval feeding rates (e.g. Shikano and Cory, 2014; Lastra et al., 1995), reduced larval weights and delays in larval development (e.g. Sedaratian et al., 2013; Janmaat et al., 2014; Gassmann et al., 2009). A proportion of a target organism population may be able to escape from or will not be susceptible to a biological control agent and larvae can potentially avoid infection through physiological or behavioural mechanisms (Williams and Hails, 1994). For example, one way that susceptibility to baculovirus infection is altered in Lepidoptera is through developmental resistance; large late instar larvae are less likely to become infected than early instars. It is possible that *Bt* could therefore influence the proportion of uninfected individuals through a slowing of larval development which in turn may delay developmental resistance i.e. larvae exposed to *Bt* are smaller and therefore more likely to consume a lethal dose of virus.

We tested the hypothesis that the co-inoculation of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) and baculovirus in an experimental Lepidoptera field population will affect the transmission dynamics of the virus by altering the proportion of uninfected larvae in the population. We investigated how this might alter primary and secondary transmission of virus when the pathogens were simultaneously or sequentially applied. We hypothesised that a reduction in the rate of host-mass gain of larvae sustaining a non-lethal *Btk* infection would reduce the acquisition of developmental resistance by those larvae to baculovirus infection. This could lead to one of two outcomes. (1) Baculovirus transmission is increased through a slowing of developmental resistance. This in turn results in many "patches" of virus (due to higher larval mortality) but each death yields lower quantities of virus from small cadavers. If number of virus patches is more important than size of patch, this will increase the probability of secondary transmission through contact/consumption by uninfected larvae. (2) Alternatively, due to the inhibitory feeding effect of *Btk* on larvae, a smaller proportion of the population are able to consume a lethal viral dose prior to virus degradation in the environment and hence primary transmission of the virus is reduced in the presence of *Btk*. Fewer viral patches may reduce secondary transmission (again assuming number of patches is more important than size of patch). If the pathogen-free refuge is affected by the presence of *Btk* in either of these ways, we further hypothesise that the presence of *Btk* will affect the quantity of baculovirus occlusion bodies available for secondary transmission through changes in occlusion body yield per cadaver and through a change in the distribution of viral inocula within the plant canopy. We tested these hypotheses in a series of caged field experiments over two years in which experimentally manipulated cabbage moth *Mamestra brassicae* L. larval populations were exposed to varying combinations of baculovirus and *Btk*.

2. Methods

2.1. Insects and pathogens

A culture of *M. brassicae* has been reared under constant controlled environment conditions (16 h light:8 h dark, 23 °C) in an insectary facility at the Centre for Ecology & Hydrology for approximately 25 years. Eggs of a known age were surface sterilised with 5% solution formaldehyde and following hatching, neonate larvae were fed on sterile semi-synthetic diet until they reached 2nd instar (approximately 4 days from egg hatch). All larvae used for experiments were actively feeding at these stages. To produce large numbers of eggs for field trials (>50,000) adult female *M. brassicae* moths were allowed to lay eggs on long strips of filter paper over 2 days. The first egg collection was kept at a lower temperature (18 °C) than the second (22 °C) so that larvae from both collections of eggs emerged on the same day. To prevent biases due to differences in egg rearing temperature, egg batches introduced to field trials were completely randomised within the experiment.

An isolate of *Panolis flammea* multiple nucleocapsid NPV (*Pafl*NPV) (Entwistle and Evans, 1987) from a natural epizootic of baculovirus in pine beauty moth *Panolis flammea* was used in all experiments. Preliminary results in the laboratory indicated that there was potentially a synergism between this virus and *Btk* which resulted in increased mortality of larvae, hence we used this particular combination of baculovirus and baterium to test this possibility under field conditions. A stock suspension of *Pafl*NPV genotype variant 4 was obtained by dosing 3rd instar *Spodoptera exigua* larvae using the diet plug feeding method (Eberle et al., 2012). Viral cadavers were collected, macerated and the resulting suspension filtered through muslin to remove large particulate matter. Viral material was purified using density gradient centrifugation (Hunter-Fujita et al., 1998) and the concentration of occlusion bodies estimated by counting in an improved bright line Neubauer haemocytometer. Viral stock was stored at −20 °C until required and dilutions were made in sterile 0.03% Tween® 20 (3 ml L^{-1}; poly(oxyethylene) sorbitan monolaurate) in distilled water. To evaluate viral yield from individual larvae, infected individuals were collected just prior to viral death, weighed and placed in individual 1.5 ml micro-tubes. Larvae were allowed to liquefy and cadavers were macerated and suspended in 1 ml of sterile