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Plant genotype and induced defenses affect the productivity of an insect-killing obligate viral pathogen

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ABSTRACT

Plant-mediated variations in the outcomes of host-pathogen interactions can strongly affect epizootics and the population dynamics of numerous species, including devastating agricultural pests such as the fall armyworm. Most studies of plant-mediated effects on insect pathogens focus on host mortality, but few have measured pathogen yield, which can affect whether or not an epizootic outbreak occurs. Insects challenged with baculoviruses on different plant species and parts can vary in levels of mortality and yield of infectious stages (occlusion bodies; OBs). We previously demonstrated that soybean genotypes and induced anti-herbivore defenses influence baculovirus infectivity. Here, we used a soybean genotype that strongly reduced baculovirus infectivity when virus was ingested on induced plants (Braxton) and another that did not reduce infectivity (Gasoy), to determine how soybean genotype and induced defenses influence OB yield and speed of kill. These are key fitness measures because baculoviruses are obligate-killing pathogens. We challenged fall armyworm, *Spodoptera frugiperda*, with the baculovirus *S. frugiperda* multi-nucleocapsid nucleopolyhedrovirus (SfMNPV) during short or long-term exposure to plant treatments (i.e., induced or non-induced genotypes). Caterpillars were either fed plant treatments only during virus ingestion (short-term exposure to foliage) or from the point of virus ingestion until death (long-term exposure). We found trade-offs of increasing OB yield with slower speed of kill and decreasing virus dose. OB yield increased more with longer time to death and decreased more with increasing virus dose after short-term feeding on Braxton compared with Gasoy. OB yield increased significantly more with time to death in larvae that fed until death on non-induced foliage than induced foliage. Moreover, fewer OBs per unit of host tissue were produced when larvae were fed induced foliage than non-induced foliage. These findings highlight the potential importance of plant effects, even at the individual plant level, on entomopathogen fitness, which may impact epizootic transmission events and host population dynamics.

1. Introduction

Changes in plants induced by microbes and/or insect herbivores can have profound effects on the interactions between the three participants, shaping their abundances and community structure (Biere and Bennett, 2013; Biere and Tack, 2013; Shikano et al., 2017a). Insect-associated microbes, whether in the gut or outside the insect body, can influence tritrophic interactions by aiding insect detoxification of phytochemicals (Adams et al., 2013; Hansen and Moran, 2014; Mason et al., 2014), enabling insects to manipulate plant physiology for their own benefit (Chung et al., 2013; Frago et al., 2012) and providing insects with essential nutrients that are unavailable in their host plants (Douglas, 2015; Hansen and Moran, 2014). Phytopathogens and soil-borne microbes can induce changes in plants that positively or negatively influence insect herbivores, through direct or indirect plant-mediated effects on the insects (Pineda et al., 2010; Tack and Dicke, 2013). Similarly, entomopathogenic fungi can endophytically colonize plants and influence plant-insect interactions potentially by changing plant defenses and growth (Ownley et al., 2010). Insect-induced changes in plants can also strongly influence the interactions between plants, insects and their pathogens (Cory and Hoover, 2006).

The ability of insect pathogens to infect their hosts is affected by variation in plant quality, both between and within plant species (Cory and Hoover, 2006). The forest tent caterpillar, *Malacosoma disstria*, was as much as 100-fold more resistant to the bacterium *Bacillus thuringiensis* when exposed to the bacterium on foliage of quaking aspen, *Populus tremuloides*, than sugar maple, *Acer saccharum* (Kouassi et al., 2001). Mortality in greenhouse whitefly, *Trialeurodes vaporariorum*, nymphs

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were significantly higher when conidial suspensions of the pathogenic fungi *Beauveria bassiana* and *Isaria fumosoroseus* were applied to cucumber plants than to tomato plants (Poprawski et al., 2000). Mortality induced by baculoviruses can also vary with the quality of the foliage ingested along with the virus, such as the variation in quality between plant species (Ali et al., 1998; Farrar and Ridgway, 2000; Hoover et al., 1998a; Keating et al., 1988), different parts within a plant (Ali et al., 1998), plant phenology (Raymond and Hails, 2007), and defensive status of the plant (i.e., constitutive vs. herbivore-induced) (Elderd et al., 2013; Hoover et al., 1998a; Shikano et al., 2017b).

While numerous studies have demonstrated plant-mediated effects on pathogen-induced mortality, several other factors can influence the population dynamics of insects and their pathogens. There are four basic population parameters that are fundamental to pathogen fitness: transmission, speed of kill, yield (number of infectious stages released from a single host) and persistence (the rate of loss of infectious stages from the environment) (Anderson and May, 1981; Raymond et al., 2005, 2002). The few studies that examined plant-mediated effects on pathogen yield focused primarily on baculoviruses (Ali et al., 2002; Cory and Myers, 2004; Hodgson et al., 2004, 2002; Raymond et al., 2002; Raymond and Hails, 2007).

Baculoviruses are food-borne insect pathogens with narrow host ranges that can cause epizootics in outbreak host populations (Cory and Myers, 2003). They are obligate pathogens, and those that infect lepidopteran insects must kill their hosts to release orally infectious stages (viral occlusion bodies; OBs) to infect new hosts. To ensure that infection occurs via horizontal transmission, a lethal dose of OBs must be consumed along with the leaf tissue on which it resides. The amount of virus that a host yields after death depends, in part, on the leaf tissue and its phytochemistry. Differential OB yields have been demonstrated with short-term feeding on different plant species at the point of virus ingestion (immediately before, during and immediately after virus exposure) (Cory and Myers, 2004; Raymond et al., 2002) and long-term feeding on different plant species, parts and host phenology (either throughout larval development or during and after virus-challenge until host death) (Ali et al., 2002; Hodgson et al., 2004, 2002; Raymond and Hails, 2007).

We recently demonstrated that short-term feeding by fall armyworms, *Spodoptera frugiperda*, at the point of virus ingestion on soybean leaf disks exhibiting induced anti-herbivore resistance, reduced levels of mortality caused by a baculovirus *S. frugiperda* multi-nucleocapsid nucleopolyhedrovirus (SfMNPV) compared to short-term exposure to non-induced leaf disks, though the speed of kill was not affected (Shikano et al., 2017b). The degree to which soybean induced resistance lowered baculovirus-induced mortality varied strongly among eight soybean genotypes (Shikano et al., 2017b). Here, we selected two soybean genotypes from our previous study, one genotype that strongly reduced lethal infection by baculovirus when induced (Braxton genotype) and another that did not have this effect (Gasoy genotype). Although records of SfMNPV epizootics in fall armyworms on soybean plants are not available, SfMNPV is known to cause epizootics in fall armyworms in pastures (Fuxa, 2004). We used the intraspecific variation in soybean-mediated effects on SfMNPV efficacy as a model system to examine how this variation may influence virus productivity. Our objectives were to determine if virus yield would be influenced by short-term exposure to different soybean genotypes and to induced and non-induced foliage, and whether long-term feeding on induced and non-induced foliage from the Braxton genotype (from virus ingestion until death) would affect virus yield. Since Braxton and Gasoy genotypes varied in defensive chemistry (phenolic content and peroxidase activity) and in their effects on fall armyworm feeding and growth, particularly when the plants were induced (Shikano et al., 2017b), we hypothesized that virus yield will be differentially affected by short-term exposure to the two plant genotypes and to induced or non-induced foliage. We also hypothesized that long-term feeding on induced soybean foliage will result in larvae producing fewer OBs at death than on non-induced foliage, in part because induced soybean defenses significantly inhibit fall armyworm growth (Shikano et al., 2017b) and smaller hosts tend to produce less virus OBs (Wilson et al., 2000). Reduced OB yield on induced foliage might also result from increased susceptibility of infected fall armyworms to induced soybean defensive chemicals, because baculovirus infection can cause a down-regulation of genes involved in detoxification and digestive function (Noland et al., 2013). Thus, infected armyworms on induced foliage may exhibit faster death than those on non-induced foliage and consequently yield fewer OBs, which is typical of the trade-off between speed of kill and OB yield exhibited by baculoviruses (e.g. Wilson et al., 2000; Redman et al., 2016).

2. Materials and methods

2.1. Plants and jasmonic acid application

Soybean, *Glycine max* (Fabaceae), seeds were obtained from the National Plant Germplasm System, United States Department of Agriculture Agricultural Research Service (USDA-ARS). Plants were grown in professional growing mix (Sunshine Mix 4 Aggregate Plus) in 10 cm plastic pots in a temperature-controlled greenhouse at 25 °C and 16L:8D supplemented with high pressure sodium lights. Plants were watered every two days, and fertilized with 3 g of Osmocote Plus (15-9-12; Scotts) when the first true leaves were fully expanded. For each experiment, 40–60 plants of each genotype were grown and randomly divided into an equal number of induced or non-induced treatments.

Gasoy 17 and Braxton genotypes were selected based on qualitative differences in jasmonic acid (JA)-induced resistance against fall armyworms and differential effects of JA-induced defenses on baculovirus efficacy (Shikano et al., 2017b). Both genotypes are from soybean maturity group VII and have determinate stem termination. Plants were exogenously sprayed with JA, as described in Shikano et al. (2017b). JA is a phytohormone that upregulates plant defenses against chewing herbivores (Thaler et al., 1996). Control plants were sprayed with the same concentration of the carrier solution. Plants were used in bioassays 48 h after JA application. Only the youngest, fully opened trifoliate leaf was used and cork borers were used to cut leaf disks (0.64 cm^2) for the experiments described below.

2.2. Insects and baculovirus

Newly molted fourth instar fall armyworm, *S. frugiperda*, were used in all experiments. The rationale for using fourth instars is provided in Elderd and Reilly (2014). To explain briefly, the initial infections before an epizootic typically occur in first instar larvae, which is the most susceptible age, through horizontal or vertical transmission. By the time infected first instars die (4–6 days) and release OBs, uninfected larvae have developed to approximately the fourth instar. Since infected fourth instars produce exponentially more OBs than infected first instars, fourth instars were the focus of this study. Eggs were obtained from Benzon Research (Carlisle, PA, USA) and larvae were reared on artificial diet (Southland Products Inc., Lake Village, AR, USA) at 29 °C and 16L:8D in 128-well insect rearing trays (Frontier Agricultural Sciences, Newark, DE, USA) until head capsule slippage in the late third instar. These larvae were transferred to individual wells of a 12-well cell culture plate without food to complete their molt. Larvae were individually weighed to the nearest 0.1 mg before use in the experiments.

Wild-type SfMNPV-B (Nicaragua) occlusion bodies (OB) were obtained from Dr. James Slavicek, USDA Forest Service (Delaware, OH). OBs were quantified under a phase contrast microscope using an improved Neubauer brightline haemocytometer.