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

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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, Spoyers,host-586.21Tfirus-

2.3. Experiment 1: Effects of short-term feeding on plants of different quality during virus-challenge on baculovirus infectivity and OB production

Please see Fig. 1 for a flowchart of the three experiments described below. To determine the effects of constitutive and JA-induced soybean defenses on baculovirus infectivity and OB production, larvae ingested foliage and virus at the same time, but consumed artificial diet before and after virus-challenge. We challenged the larvae with SfMNPV as described in Shikano et al. (2017b). Briefly, comparable numbers of leaf disks were removed from each Braxton and Gasoy plant, which had been JA- or control-treated, and equally distributed to each virus dose. There were 30 leaf disks per dose for each induction treatment per genotype. Leaf disks were kept moist on wet paper towels. A 2 μl droplet of Milli-Q water containing one of fi

number of days to initiate pupation and pupal weights of control insects were also measured. Two control larvae initiated pupation but failed to eclose fully to the pupa, and thus, these larvae were included in time to pupation analysis but not pupal weight.

2.5. Experiment 3: Effects of long-term feeding on JA-induced plants on OB production and speed of kill

To determine whether the plant treatment consumed after virus-challenge would impact OB production and speed of kill, we challenged larvae with the highest virus dose used in experiment 1 (30,000 OBs) on JA-induced or non-induced leaf disks, and subsequently reared them until death or pupation on foliage from their respective plant treatments. Foliage from each plant treatment was provided to each larva in individual 30 ml plastic cups lined with moist paper towel. Plants were treated with JA or a control solution in two-day intervals, such that larvae could be provided with new foliage every two days. There were 36 virus-challenged larvae and 9 control larvae per plant treatment. The number of days to initiate pupation and pupal weights of control insects were measured. Mortality was recorded, cadavers were weighed, and the numbers of OBs per cadaver were counted. There was one virus-killed cadaver that was weighed, but the OB count was missing.

2.6. Statistical analyses

Experiment 1. We analyzed the dose response mortality of larvae that ingested virus on JA-induced or non-induced leaf disks from the Braxton and Gasoy soybean genotypes together using a generalized linear model (GLM; binomial error distribution and logit link function). The mortality data for Braxton and Gasoy genotypes were previously included as part of a larger-scale dose response analysis on five genotypes in the supplementary materials of Shikano et al. (2017b). Time to death and OB yield measurements were collected from the dose response assays, but were not reported in Shikano et al. (2017b). Time to death was analyzed using a Cox proportional hazards model and OB yield was analyzed by analysis of covariance (ANCOVA). The lowest two doses from each plant treatment were excluded from time to death and OB yield analyses due to low sample sizes (< 10 dead larvae). Soybean genotypes, induction treatment and $\log_{10}$dose, and their interactions were included as predictor variables. Differences among plant treatments in the relationship between OB yield and speed of kill were analyzed using multiple linear regression (MLR). OB yield was the response variable and time to death, $\log_{10}$dose, plant genotype and induction treatment and their interactions were included as predictor variables.

Experiments 2 and 3. An ANCOVA was used to determine differences in the OB yield and cadaver weight. Difference in pupal weight of control insects was also analyzed by ANCOVA. Time to death of virus-challenged larvae and days to pupation of control larvae were analyzed by Cox proportional hazards. The difference in development rate in the first 24 h after ingesting a JA-induced or non-induced leaf disk was determined by comparing the proportion of larvae that were still in the fourth instar, as opposed to the molting or fifth instar stages, using GLM with Firth bias-adjusted estimates. The change in OB yield with cadaver weight (virus efficiency) was analyzed by MLR with OB yield as the response variable and the plant induction treatment and cadaver weight as predictor variables. The relationship between OB yield and speed of kill was analyzed by MLR with OB yield as the response variable and the plant induction treatment and time to death as predictor variables. For all of the analyses used in the three experiments, the initial larval weight at the time of virus-challenge was included as a covariate. Sex was also included as a covariate for pupal weight analyses. All non-significant interactions were sequentially removed to produce the final minimal model. JMP Pro 12 (SAS Institute, Cary, NC, USA) was used for all analyses.

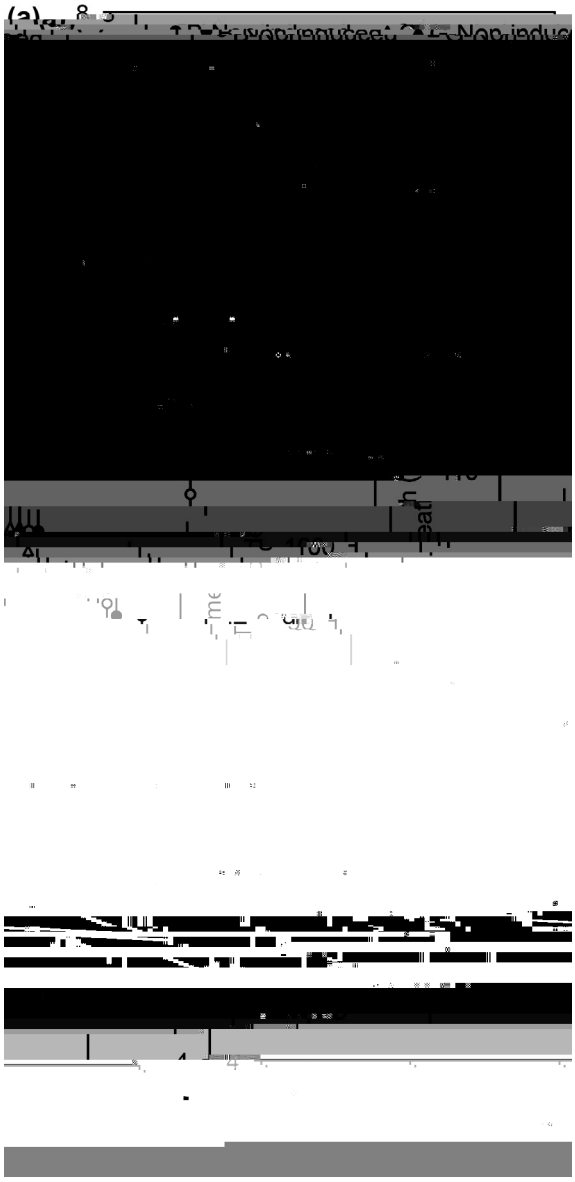

Fig. 2. (a) Logit mortality, (b) time to death and (c) OB yield of larvae challenged with a range of SfMNPV doses on JA-induced or non-induced leaf disks from Braxton or Gasoy soybean genotypes (short-term feeding on plant treatments; experiment 1). Five doses were used to determine logit mortality. Only the highest three doses were used for time to death and OB yield analyses because the two low doses killed fewer than 10 hosts in each plant treatment. Fitted lines represent final minimal models fitted by (a) GLM and (c) ANCOVA. Black lines represent Braxton, grey lines represent Gasoy, solid lines represent non-induced and dashed lines represent JA-induced. No line is available for (b) Cox proportional hazards, though time to death decreased significantly with increasing dose. Symbols are shifted along the x-axis to prevent overlap.

3. Results

3.1. Short-term feeding on different quality plants during virus-challenge influenced baculovirus infectivity and OB production (experiment 1)

Mortality of fall armyworm by SfMNPV responded differently to JA-induced defenses in the two soybean genotypes (Fig. 2a; Table 1; genotype by plant induction, $X^2_1 = 3.86$, $p = 0.049$). Virus-induced mortality was significantly lower when the virus was ingested with JA-induced leaf disks than with non-induced leaf disks on the Braxton genotype (paired contrast, $X^2_1 = 16.60$, $p < 0.0001$), but there was no effect of JA-induction on the Gasoy genotype (paired contrast, $X^2_1 = 1.71$, $p = 0.19$). Virus-induced mortality was not significantly

different between the two genotypes if the leaf disks were not induced (i.e. constitutive plant defenses; paired contrast, $X^2_1 = 0.40$, $p = 0.53$), but mortality was significantly lower for Braxton when both genotypes were JA-induced (paired contrast, $X^2_1 = 11.72$, $p < 0.001$). As expected, mortality increased with virus dose ($X^2_1 = 270.53$, $p < 0.0001$). No other interactions between plant induction, genotype and dose were significant.

Virus-challenged armyworms died faster as the dose increased ($X^2_1 = 10.94$, $p < 0.001$; Fig. 2b), but this response was not affected by plant induction ($X^2_1 = 1.83$, $p = 0.18$) or soybean genotype ($X^2_1 = 0.87$, $p = 0.35$). All other interactions between plant treatment, genotype and dose were non-significant.

The number of OBs produced per cadaver decreased with increasing virus dose (F_{22}

3.7. Effects of short and long-term feeding on JA-induced plants on larval development

Control larvae (unchallenged larvae from experiment 2) tended to develop more slowly in the first 24 h on artificial diet following the ingestion of a JA-induced leaf disk compared to a non-induced leaf disk (Fig. 5a; $X^2_1 = 3.24$, $p = 0.072$). However, this short-term exposure to the plant treatments had no significant impact on time to pupation (Fig. 5b; $X^2_1 = 0.22$, $p = 0.64$) or pupal weight (Fig. 5c; $F_{1,37} = 0.67$, $p = 0.42$).

Control larvae (unchallenged larvae from experiment 3) developed slower in the first 24 h on JA-induced foliage following the ingestion of a JA-induced leaf disk compared to larvae that fed on non-induced foliage after ingesting a non-induced leaf disk (Fig. 5a; $X^2_1 = 6.85$, $p = 0.009$). Larvae that were fed JA-induced foliage after a JA-induced leaf disk took 40% longer to reach pupation (Fig. 5b; $X^2_1 = 8.40$, $p = 0.004$) and produced pupae that were 27% lighter than larvae that fed on non-induced leaf disk and foliage (Fig. 5c; $F_{1,14} = 36.51$, $p < 0.0001$).

4. D

In this study, we used the Braxton soybean genotype, which lowered

this scenario, our findings suggest that induced plant defenses may limit the number of OBs released for the next round of horizontal transmis-

With only a handful of studies that have examined plant-mediated

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