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Volatiles that encode host-plant quality in the grapevine moth

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ABSTRACT

Plant volatiles are signals used by herbivorous insects to locate host plants and select oviposition sites. Whether such volatiles are used as indicators of plant quality by adult insects in search of host plants has been rarely tested. We tested whether volatiles indicate plant quality by studying the oviposition of the grapevine moth *Lobesia botrana* on the grapevine plant *Vitis vinifera*. Host plants were infected with a variety of microorganisms, and larval fitness was correlated to the infected state of the substrate. Our results show an oviposition preference for volatiles that is significantly correlated with the fitness of the substrate. The chemical profiles of the bouquets from each *V. vinifera*–microorganism system are clearly differentiated in a PCA analysis. Both the volatile signal and the quality of the plant as larval food were affected by the introduction of microorganisms. Our study represents a broad approach to the study of plant–insect interactions by considering not only the direct effect of the plant but also the effect of plant–microorganism interactions on insect population dynamics.

1. Introduction

Plant–insect interactions are mediated by plant chemistry. Female herbivorous insects use sensory cues to locate and select a suitable host plant for egg laying (Schoonhoven et al., 2005). Olfaction, contact chemoreception and vision are involved in this process (Chapman, 2003; Hilker and Meiners, 2002; Tasin et al., 2011). In contrast to vision and contact, olfactory cues can be detected at a considerable distance from the source (Bruce et al., 2005). The olfactory profiles of host plants can be modified by a number of factors and may reflect changes in host quality. Host odor is comprised of a number of volatile organic compounds (VOCs) in a specific blend ratio (Knudsen et al., 1993). Variation in host quality may affect the composition of these odors, and this change may be perceived by the olfactory apparatus of insects in search of hosts (Agosta, 2006; Dotterl et al., 2009; Najar-Rodriguez et al., 2010; Tasin et al., 2006). In insects with a non-feeding adult stage, the quality of food consumed in the larval stage determines reproductive performance. The reproductive potential of these species is stringently associated with the accumulation of resources during the larval stage (Awmack and Leather, 2002). A body of the literature has investigated the response of insects to variation in host quality at both the individual and population levels (Agosta, 2008; Diamond et al., 2010; Franzke et al., 2010; Mayhew, 2001), and attention has been paid to the mechanism behind host/food choice by egg-laying females for their offspring. Factors affecting this choice may be of crucial importance for the survival and fitness of the species (Gripenberg et al., 2010).

Plants emit a number of volatile secondary metabolites. Insects perceive a small subset of these compounds, but the role of plant-released VOCs as signals to associated herbivores has not been fully elucidated. In some herbivorous species of moths, olfactory cues released from hosts are a major stimulus, both in guiding gravid females to potential oviposition substrates and in triggering oviposition at the host (Bruce et al., 2005; Renwick and Chew, 1994). The behavioral role of these compounds has been qualitatively and quantitatively studied with regard to odor profile and behavioral plasticity (Anton et al., 2007; Tasin et al., 2007). However, an investigation into the role of host plant volatiles as an indicator of larval food quality has rarely been attempted in moths (Takacs et al., 2001).

Microorganisms are known to affect the nutritional value of host plants, leading to positive, neutral or detrimental effects on the fitness of larger insect herbivores. Microorganisms may also modify plant odor profiles (Cosse et al., 1994; Drilling and Dettner, 2009). The effect of plant–microorganism interactions on the population dynamics of herbivorous insects has not been exhaustively considered by studies that examined plants or microorganisms alone (Hatcher, 1995).

In vineyards, gravid females of herbivorous insects are confronted with a diverse range of food sources, including grapes that are either healthy or colonized with a diverse range of yeast, bacteria and fungi. In this study, we hypothesized that herbivorous insects use the volatile signal from a host plant to assess plant food quality for its offspring. This hypothesis was tested by studying the

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oviposition of the grapevine moth *Lobesia botrana* (Denis and Schiffermueller) (Lepidoptera: Tortricidae), on the grapevine plant *Vitis vinifera* L. (Vitacae). Plants were infected with a range of microorganisms (a consortium of yeast: *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, *Zygosaccharomyces rouxii* (Boutroux) Yarrow, *Metschnikowia pulcherrima* (Pitt.) M.W. Miller, *Hanseniaspora uvarum* (Niehaus) and *Pichia anomala*; a consortium of bacteria: *Gluconobacter oxydans* (Henneberg) De Ley and *Acetobacter aceti* (Pasteur) Beijerinck; a fungus: *Botrytis cinerea* Pers.), endemic of grape surface. We compared moth oviposition preference for infected grapes with the fitness of larvae on a diet of infected food. In addition, we analyzed the odor profiles emitted by the food sources. We attempted to find an association between behavioral response and the odor composition of the food source using principal component analysis.

2. Results

2.1. Larval and pupal traits

Males reared on a diet to which fungus had been added developed significantly slower than males reared on other diets (Table 1). The time between egg and pupae stages was shorter for males than for females. Each microorganism differentially affected pupal mass. Female pupae fed on a yeast diet were heavier than those fed on the base diet. In contrast, the diet with *B. cinerea* produced lighter male pupae (Table 1). A higher proportion of larvae pupated on yeast- or bacteria-enriched diets; no such difference was recorded between the base and *B. cinerea*-enriched diets (Table 1). The percent emergence from the four diets did not differ significantly (Table 1).

2.2. Adult performance

The addition of microorganisms to the base diet had no effect on the sex ratio of adults, the length of the period prior to egg-laying, the duration of egg-laying, or the fecundity and longevity of egg-laying females (Table 1). The proportion of mated females was significantly increased by the addition of the three powdered microorganisms, as compared with the base diet (Table 1). Yeast- or bacteria-enriched diets augmented female fertility as compared with the base diet, but there was no distinguishable effect of *B. cinerea* on this parameter. Insects that were reared on yeast- or bacteria-enriched diets showed a higher female survival rate into the next generation, as well as a higher fitness index than insects developed on the base diet or a diet containing phytopathogenic fungus (Table 1).

2.3. Analysis of microbial volatiles

We detected 27 volatile compounds in the profile of grapes infected with yeast, bacteria and *B. cinerea*. The odor composition varied greatly with microorganism type (Table 2). The average amount of ethanol collected with our SPME method ranged from 6.3 (bacteria) to 7.6 (yeast) to 9.1 (fungus) μg/g of grape biomass. The odor profile released by the three grape-microorganism systems differed significantly (50–50 MANOVA, df = 2,6; $P < 0.001$; $N = 3$; $\chi^2 = 75.54$; N of molecules in the model = 27), as represented by the PCA shown in Fig. 1. Ethanol, 3-methyl-1-butanol and ethyl acetate were, in characteristic and significantly different proportions, the main components in grapes infected with fungi and yeast. These compounds, together with acetic acid, were also the main volatiles in the bacteria-infected odor profile (Table 2). Some esters, such as methyl acetate, isobutyl acetate and isoamyl acetate, were only detected in the yeast odor. Isovaleric acid was only found as typical component of the bacteria-infected volatile profile. All of the compounds emitted by the fungus-infected grapes were detected in at least one other infected sample.

2.4. Adult preference test

The odors released by *V. vinifera* infected with *B. cinerea* were repellent to egg-laying *L. botrana* females: only 28% of eggs were deposited on the fungus-infected side (2.3 (fungus) vs. 5.7 (uninfected) eggs/female). In contrast, we observed a preference for the odor released by *V. vinifera* infected with yeast (66%; 7.8 (yeast) vs. 4.2 (uninfected) eggs/female). An intermediate but repellent response was measured for the odor emitted by bacteria-infected plants (37%; 3.8 (bacteria) vs. 6.5 (uninfected) eggs/female).

Table 1
Effects of larval food on life-history traits of *Lobesia botrana*. One hundred neonate larvae were used per diet treatment.

Life-history traits (±SEM)	Larval food				Statistics	
	Semisynthetic diet (B)	B + Yeast	B + Acetic acid bacteria	B + *Botrytis cinerea*	χ^2	*P*
Development time (d)						
Female	47.8 ± 1.6	43.4 ± 0.9	43.9 ± 1.8	46.8 ± 1.3	8.7[a,c]	0.03
Male	41.8 ± 0.8 AB	41.1 ± 0.7 A	39.7 ± 1.6 A	45.1 ± 1.4 B	10.0[a,c]	0.02
Pupal mass (mg)						
Female	12.4 ± 0.6 B	14.3 ± 0.4 A	13.7 ± 0.5 AB	13.6 ± 0.6 AB	8.6[a,c]	0.04
Male	10 ± 0.2 AB	10.5 ± 0.2 A	10.5 ± 0.3 A	9.4 ± 0.3 B	13.2[a,c]	0.004
Pupation (%)	9. B	34.1 A	28.6 A	11.5 B	160.6[b,d]	<0.001
Adult emergence (%)	88.6	83.1	79.5	76.5	3.9[b]	0.272
Female sex ratio (%)	40.0 ± 5.9	43 ± 4.4	48.4 ± 6.4	42.5 ± 7.9	0.9	0.81
Mated females (%)	50 ± 9.3 B	79.6 ± 5.5 A	78.6 ± 7.9 A	81.0 ± 8.8 A	10.3[a,d]	0·02
Delay in egg-laying (d)	4.2 ± 0.5	3.5 ± 0.3	4.2 ± 0.3	4.7 ± 0.6	7.1	0.07
Duration of egg-laying (d)	2.9 ± 0.9	3.2 ± 0.2	2.9 ± 0.3	2.7 ± 0.4	1.9	0.59
Fecundity (no. eggs)	62.2 ± 17.4	106.5 ± 11.5	95 ± 15.9	87.6 ± 18.8	5.9	0.11
Fertility (no. of viable eggs)	28.3 ± 8.2 B	54.8 ± 4.2 A	68.5 ± 9 A	47.8 ± 7.1 AB	14.8[a,d]	0.002
Longevity of egg-laying females (d)	9.0 ± 0.9	9.2 ± 0.6	9.1 ± 0.8	9.9 ± 2.4	0.44	0.93
Larvae produced per mated female (n)	38.3 ± 13.3 B	76.4 ± 9.2 A	82.2 ± 13.6 A	52.8 ± 11.4 A	10.9[a,c]	0.012
Females produced (n)	1.1 ± 0.4 B	9.3 ± 1.1 A	9 ± 1.5 A	2 ± 0.4 B	29.8[a,c]	<0.001
Fitness index (Female produced × % Mated females)	0.6 ± 0.2 B	7.4 ± 0.9 A	7.1 ± 1.2 A	1.6 ± 0.4 B	31.0[a,c]	<0.001

[a] Kruskal–Wallis ANOVA.
[b] 2 × N Chi square on numbers of pupae and adults.
[c] Steel–Dwass test for non-parametric multicomparison.
[d] Ryan multicomparison test for proportion.