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Oviposition behaviour and life-history performance of *Epiphyas postvittana* (Lepidoptera: Tortricidae) on the *leaves of Vitis vinifera* (Vitales: Vitaceae) infected with *Botrytis cinerea* (Helotiales: Sclerotiniaceae)

S. Z. M. Rizvi and A. Raman

Charles Sturt University & Graham Centre for Agricultural Innovation, Orange, Australia

Summary

In three-way interaction systems involving an insect and a plant-pathogenic fungus, both occurring on the same plant, the insects generally gain in terms of their growth and metabolism. In this study we have tested how the infection by *Botrytis cinerea* on the leaves of *Vitis vinifera* influences the life-history performance of larvae and the oviposition behaviour of *Epiphyas postvittana*. We conducted free-choice and two-choice experiments to test the oviposition behaviour of gravid *E. postvittana*. We also characterized the effects of *B. cinerea*-infected leaves of *V. vinifera* on the growth and development of *E. postvittana*. We found that the oviposition preference of *E. postvittana* was strongly influenced by the olfactory and tactile cues. Volatiles from *B. cinerea*-infected plants significantly deterred oviposition and in consequence, adult females laid fewer number of eggs on *B. cinerea*-infected leaves of *V. vinifera* compared with uninfected leaves. The mortality rate of larvae fed on *B. cinerea*-infected leaves were not significantly different from the larvae fed on uninfected leaves of *V. vinifera.* Whereas, the larvae of *E. postvittana* fed on *B. cinerea*-infected leaves had significantly shorter developmental period, attained heavier pupal mass, and on becoming adults they laid more numbers of eggs than the larvae that were enabled to feed on uninfected leaves of *V. vinifera.* We also reared the larvae of *E. postvittana* on exclusive-fungus diet but all larvae died before pupation indicating that for a better larval performance and adult reproductive output of *E. postvittana*, the *V. vinifera-B. cinerea* interacting system is but imperative.

K e y w o r d s : grapevine; larval development; light-brown apple moth; oviposition deterrence; three-way interaction.

Introduction

Infection by pathogenic fungi alters the chemistry of host plants and influences oviposition preference and larval performance in plant-feeding insects (Mondy and Corio-Costet 2000, Rizvi *et al*. 2015). Fungal infections can interfere with the biosynthetic pathways in the plant in various ways. Some lead to a decrease in the production of insect-attracting volatiles (Dötterl *et al*. 2009), whereas others produce several behaviour-modifying volatiles (Tasin *et al.* 2011). Fungal infection of a plant can suppress its direct defence capability against plant-feeding insects by modifying the plant's secondary metabolism (Thaler *et al*. 1999) and the nutritional quality of the plant, thus rendering the plant susceptible for insect colonization (Cardoza *et al*. 2003). On the other hand, fungal infection can deteriorate the nutritional quality of the plant and influence the plant-feeding insect's development negatively (Tasin *et al*. 2012).

Olfactory cues are critical for searching a suitable host from a distance for oviposition but once the insect lands on the target plant, the post-landing cues, viz., contact, together with the volatiles can influence the insect for an assessment of the plant and can either deter or stimulate oviposition (Renwick and Chew 1994, Foster *et al*. 1997, Tasin *et al*. 2011). Based on the sensory cues of a plant-feeding insect, judgment on the appropriateness of a host plant is essential for the success of progeny performance (Gripenberg *et al*. 2010). Optimization theory of host searching suggests that the gravid female should choose those plants, which maximizes the fitness of her progeny (Jaenike 1978), although evidences negating Jaenike (1978) also exist (Larsson and Ekbom 1995, Leyva *et al.* 2000). The choice made by juveniles of insects for food can substantially differ from that made by adults, particularly when the larvae and adults feed on different plant parts (Mayhew 1997). In the natural environment, several organisms attack plants; among these, insects and fungi are associated with a majority of plants, establishing a three-way interacting system. In such contexts, the fungi influence the interaction between the insect and the plant, which can be either mutualistic or antagonistic, and, occasionally, neutral as well (Hatcher 1995). The fungi, in such interacting systems, can induce multiple variations in plant chemistry that can be recognized by plant-feeding insects by their olfactory capacity which, in turn, can modify the preference behaviour in insects (Najar-Rodriguez *et al*. 2010)

Nevertheless, in three-way interacting systems involving an insect and a plant-pathogenic fungus, both developing on the same plant, the insects generally gain in terms of their growth and metabolism. Leaf tissues of *Arachis hypogaea* (Fabales: Fabaceae)-infected with *Sclerotium rolfsii* (Atheliales: Atheliaceae) showed significantly greater levels of soluble sugars but lower starch content and total soluble phenolics compared with uninfected leaves of *A. hypogaea*. *Spodoptera exigua* (Lepidoptera: Noctuidae) showed significantly greater survival rates, produced significantly heavier pupae, and had shorter time span be-

Correspondence to: Dr. A. Raman, Charles Sturt University, PO Box 883, Orange, NSW 2800, Australia. Fax: +61-2-6365-7833.
E-mail: araman@csu.edu.au

tween the last larval instar and pupation, when fed on the foliage of *A. hypogaea* infected with *S. rolfsii* (Cardoza *et al*. 2003). For instance, in the *Botrytis cinerea-Lobesia botrana* (Lepidoptera: Tortricidae)-*V. vinifera* interacting system, *L. botrana* have been demonstrated to gain from *B. cinerea* by acquiring sterols, which enhanced their ability to metamorphose rapidly gaining greater biomass (Mondy and Corio-Costet 2000). The nutrient composition and levels of fungal infection in plants influence the fitness and reproductive performance of an insect (Biere and Tack 2013). These findings indicate that the Lepidoptera gain from fungi associated with plants, as demonstrated by accelerated growth rate, although in essence the insect can feed only on plant tissue and still perform.

Keeping the above in view, we used a three-way interacting system of *Epiphyas postvittana-Vitis vinifera-Botrytis cinerea* and proposed that *E. postvittana* co-occurs with *V. vinifera* infected by *B. cinerea*, and that for a better larval performance and oviposition behaviour of *E. postvittana*, *V. vinifera-B. cinerea* interacting system is imperative. *Epiphyas postvittana*, an Australian native Tortricidae, damages leaves and fruits of *V. vinifera* grown in Australasia extensively. This species was accidentally introduced into England, Ireland, the Netherlands, Sweden, besides Hawaii and California of USA (Suckling and Brockerhoff 2010). Field evidences and laboratory studies indicate that the larvae of *E. postvittana* transmit the conidia of *B. cinerea* (Rizvi *et al*. 2015). *Botrytis cinerea* is an opportunistic necrotrophic pathogen that induces grey-mould disease on the leaves and fruits of *V. vinifera* (Fournier *et al*. 2013).

In this study, we sought answers to the following questions: (1) whether the females of *E. postvittana* prefer ovipositing on *V. vinifera* infected by *B. cinerea,* (2) whether *B. cinerea*-infected leaves positively influence larval performance of *E. postvittana,* and (3) whether the larvae of *E. postvittana* can survive and develop feeding exclusively on *B. cinerea*. To secure answers, we tested the oviposition preference of *E. postvittana* using *B. cinerea*-infected and uninfected leaves of *V. vinifera.* To test the life-history performance with or without *B. cinerea*, we raised the larvae of *E. postvittana* on uninfected and *B. cinerea*-infected *V. vinifera* leaves, using standard plant-nutrient solution (e.g., Knop's solution) as well as on standard synthetic culture media (viz., potato-dextrose agar [PDA], Murashige and Skoog medium [M-S medium]). To verify the survival and development rate of the larvae of *E. postvittana*, exclusively on *B. cinerea* we reared the larvae on *B. cinerea* cultured on PDA and M-S medium, without *V. vinifera*.

Material and Methods

Insect culture: The larvae of *E. postvittana* were cultured on a *Phaseolus lunatus*-based semi-synthetic diet (Cunningham 2007) and maintained at 21 ± 1 °C, 60-80 % RH, and 16L: 8D regimen. On emergence, an adult female was placed with a similar-aged adult male to facilitate mating as described previously (Rizvi *et al.* 2015). All eggs (5-6 d old) were surface-sterilized using 5 % formaldehyde solution and then washed with sterilized water following Raman and Beiderbeck (1992).

Fungus culture and preparation of conidial suspension: *Botrytis cinerea* isolated from infected berries of *V. vinifera* 'Chardonnay' from the CSU-O's 'Chardonnay' vineyard in February 2013 was used. Stock culture was maintained on PDA (Fisher Scientific, Inc., Scoresby, Victoria, Australia) at 22 °C and 12L: 12D. The conidial suspension was prepared in sterile water following Rizvi *et al*. (2015) and adjusted to 10^6 conidia·mL^{-1}.

Inoculation of *V. vinifera* leaves with *B. cinerea*: Sixty plants of *V. vinifera* 'Chardonnay' (hereafter *V. vinifera*) were raised in polypropylene pots (17 cm tall, 10 cm Ø) containing commercial potting mix (Osmocote, Plus Organics Vegetable & Herb Mix, Sydney, Australia) in an insect-proof glasshouse at 23 ± 2 °C, 60 % RH, and 16L: 8D. Six-week old plants were used in the trials. The conidial suspension was sprayed using a hand-held atomizer to infect the leaves of *V. vinifera*. Uninfected (control) leaves were sprayed with sterile water identically. The inoculated and uninoculated plants were covered with a polyethylene bag to enhance humidity and placed in an environmental chamber (Thermoline L+M, Thermoline Scientific, NSW, Australia) (23 °C, 70 % RH, and 16L: 8D). Those plants which manifested disease symptoms were used in the experiments. The disease symptoms on *V. vinifera* leaves were estimated by measuring in percentage by following (Rizvi *et al.* 2014a). Those plants which showed 10-30 % infection level were used in the oviposition bioassays.

Oviposition behaviour

Free-choice experiment: Three uninfected and three *B. cinerea*-infected plants were placed in an aluminium-mesh cage (200 x 80 x 80 cm^3). The plants were placed 10 cm away from the edge of the cage and were distributed in two rows, separated by 40 cm between plants. Uninfected and infected plants were placed alternately (Fig. 1). Ten gravid females of *E. postvittana* were released at the midpoint of the cage allowing the insects to choose either *B. cinerea*-infected or uninfected plants for oviposition. The setup was left undisturbed for 72 h. After 72 h, the plants were verified for eggs and the number of eggs was counted under a stereo-binocular microscope (S-20, AIS Instrument Services, Croydon, Victoria, Australia). This experiment was repeated six times. In each replicate new plants were used and the position of the infected and uninfected plants were randomized.

Two-choice experiment: One *B. cinerea*-infected and one uninfected plants were individually placed in two glass containers (60 x 30 x 30 cm^3), which had tiny holes on the one side to facilitate the passage of air. The two containers were connected by a horizontal glass tube (30 cm long, 3 cm Ø). At the mid-point of the horizontal glass tube, a 2-cm wide circular port was cut to introduce the moths (Fig. 2). Five gravid individuals of *E. postvittana* were introduced through the port, enabling them to choose either *B. cinerea*-infected or uninfected plant for oviposition. Soon after introduction, the port was closed using a Parafilm® strip. After 72 h, the insects were removed. The choices made by the insects were recorded and the numbers of eggs laid on *B. cinerea*-infected and uninfected *V. vi-*

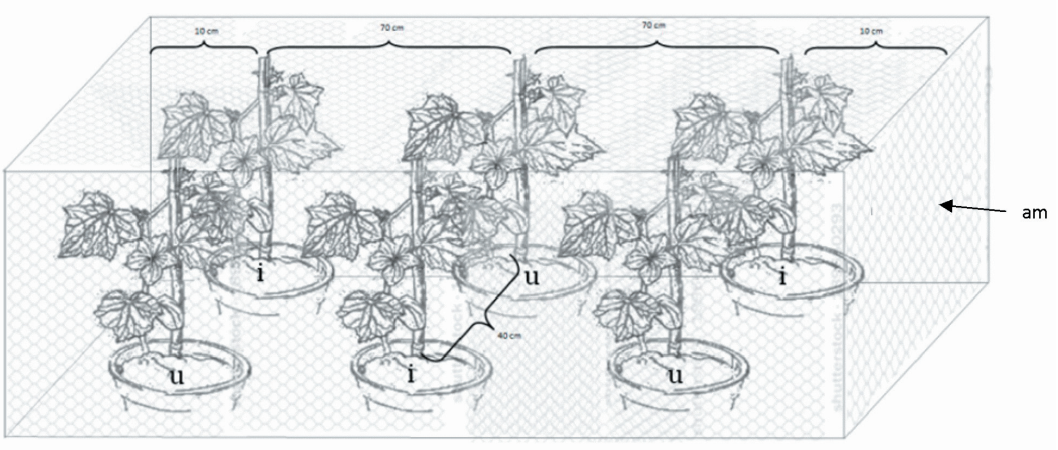

Fig. 1: Custom-made aluminium cage made for the free-choice experiment of adults of *Epiphyas postvittana* (not to scale) (am: aluminium mesh, i: infected plant, u: uninfected plant).

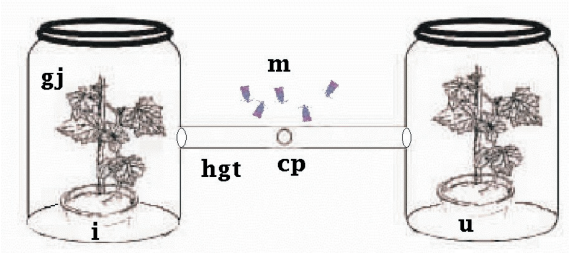

Fig. 2: Custom-made glass device for the two-choice experiment using adults of *Epiphyas postvittana* (not to scale; cp: circular port, gj: glass jar, hgt: horizontal glass tube, ip: infected plant, m: moth, p: Parafilm, up: uninfected plant).

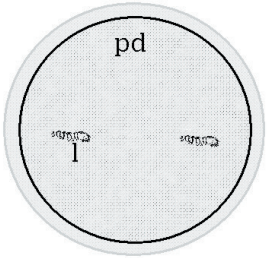

Fig. 3: Petri-dish pair for the development of the larvae of *E. postvittana* on *B. cinerea.* (not to scale; l: larva, pd: Petri dish, l: Petri dish lid).

nifera were counted under a stereo-binocular microscope. This experiment was repeated six times. In each replicate new plants were used and the position of the infected and uninfected plants were randomized.

Larval development

On *B. cinerea* cultured on synthetic media: Thirty Petrie plates (9 cm Ø) of PDA were inoculated using a 4 mm^2 PDA block from stock culture of *B. cinerea*. These plates were incubated at 21 °C, 12L: 12D. Thirty sterile PDA plates were used as control (Fig. 3). After 7 d, ensuring that *B. cinerea* was growing in the newly introduced plates, the 4 mm^2 PDA block was removed. Two neonate larvae (< 4 h after emergence) from sterile source were placed in every inoculated and sterile PDA plates, which were sealed immediately using Parafilm. The plates were then incubated at 21 °C, 12L: 12D. An identical procedure was applied to raise the larvae of *E. postvittana* on aseptically cultured *B. cinerea* on M-S medium (Murashige and Skoog 1972). All procedures were conducted in the clean-air environment of the horizontal laminar-airflow bench (HWS120, Clyde-Apac, Sydney, Australia).

On *B. cinerea*-infected and uninfected leaves of *V. vinifera* maintained on nutrient medium and on synthetic media : From freshly obtained branches of glasshouse-raised *V. vinifera*, several 20-cm stem segments bearing 5-6 fully unfurled leaves were excised for use in this experiment. The stem segments were surface-sterilized using 1 % NaClO solution for 5 min and rinsed in sterile water (3x). The leaves on these excised stem segments were then sprayed with the spore suspension in a laminar-airflow cabinet. The leaves on stem segments used as control were sprayed with sterile water. The basal tip of each segment was fixed on an Oasis® floral foam (4 cm^3) soaked in Knop's solution (Rychter and Mikulska 1990). The inoculated and uninoculated leaf-bearing stem segments were placed in a zip-lock plastic bag (35x40 cm^2), which included a damp-filter paper. The set up was maintained at 22 °C and 12L: 12D for 4-5 d.

Only those leaves bearing around 10 % infection level were used in this experiment. The stem segments bearing either infected or uninfected leaves were placed separately in glass jars (22 cm tall, 15 cm Ø) (Fig. 4). Two neonate larvae (< 4 h after emergence) were placed on infected and uninfected leaves and maintained at 21 ± 1 °C, 60-70 % RH, and 16L: 8D. After every 4 d, the stem segments were changed to maintain the infection level under 30 % of leaf area and frass was removed from the plate. Fifty larvae were used for each treatment. Data pertaining to the rate of survival of the larvae were collected every 4 d. The dates of pupation, number of pupae, and pupal mass were recorded. Pupal mass was used as an indicator of adult-body mass, because adult body mass cannot be obtained without killing them. Each pupa was quarantined to a stoppered glass vial (4 cm tall, 2 cm Ø) until emergence. Adult emergence and sex ratio (%) were recorded. The adults were sexed and paired in a corrugated-wall Dixie cup enabling mating and oviposition. The adults enabled to mate and oviposit

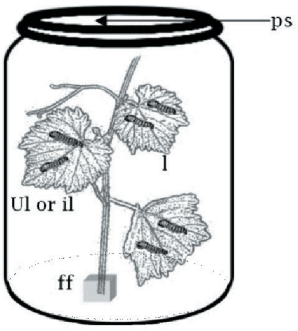

Fig. 4: Custom-made glass device for the rearing of the larvae of *Epiphyas postvittana* (not to scale; ff: floral foam, il: infected leaf, l: larva, ps: parafilm seal, ul: uninfected leaf).

were subsequently measured for their performance: fecundity rate, possible delays in oviposition (measured in days), viability (measured as the number of emerged larvae). The eggs were incubated at 21 ± 1 °C, 60-70 % RH, and 16L: 8D for 15 d enabling larval emergence. The number of days from the date of emergence of adults to death was also recorded. Life-history performance of *E. postvittana* on *B. cinerea*-infected or uninfected leaf was further verified through using two aseptic nutrient media. Excised surface-sterilized leaf (petioles+laminae) of *V. vinifera* was fixed onto either the PDA or M-S medium in a test tube (10 cm long, 1.5 cm Ø, sealed with Parafilm) by pushing the petiole through the solid medium (Fig. 5). Leaves were sprayed with spore suspension, whereas sterile water sprays were used in control. All work was done on laminar-airflow bench, maintained at 22 °C and 12L: 12D.

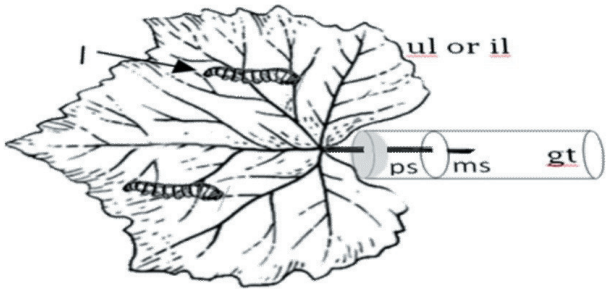

Fig. 5: Custom-made glass device for the rearing of the larvae of *Epiphyas postvittana* (not to scale; l: larva, gt: glass tube, il: infected leaf, ms: medium slant, ps: Parafilm seal, ul: uninfected leaf).

Leaves with 10 % infection were used for rearing the larvae of *E. postvittana.* Two larvae were released on each leaf. Every 4 d, the survival rate of larvae were noted and larvae were shifted onto either a new infected or a new uninfected leaf set up in the PDA or M-S medium as described above. The larvae were allowed to pupate and the life-history traits were noted. Fifty larvae were used for each treatment. Aseptic culture system has provided an environment to experimentally verify the life-history performance of many plant feeding insects. One of the offshoots of such trials is raising the plant-feeding arthropods on their host plants in control environment (e.g. Forneck *et al*. 1998; Forneck *et al*. 1999).

Statistical analysis : In the oviposition bioassay, the numbers of eggs laid on *B. cinerea*-infected and uninfected leaves in free-choice experiment and two-choice experiment were analyzed using paired sample "t"-test. Linear regression was applied to discriminate the significant difference in the mortality rate of larvae applying "$y = a + bx$", where x is the day and y is the mortality. A contingency table (χ^2) was used to analyze female sex ratio, survival percentage of larvae from egg hatching to pupae, and survival percentage of pupae from pupation to adult emergence. Independent sample t-test was used to analyse all other life-history traits. Analyses were made with SPSS statistic 17.0 (1993-2007) and GenStat (VSN International 2012). Graphs were generated in MS Excel 2013 and GenStat.

Results

Oviposition behaviour

Free-choice experiment: *Epiphyas postvittana* females deposited significantly more number of eggs on uninfected leaves than on *B. cinerea*-infected leaves (uninfected *vs.* infected, 732.6 ± 59.44 *vs.* 236 ± 40.5, t = 13.91, $p < 0.001$, Fig. 6).

Two-choice experiment: Gravid females of *E. postvittana* showed a significant preference for uninfected leaves (73.7 %) as against the infected leaves (26.3 %, $n = 30$, $\chi^2 = 6.53$, $p < 0.05$, Fig. 7). The *B. cinerea*-infected leaves significantly inhibited oviposition, compared with the uninfected leaves (uninfected *vs.* infected, 399 ± 64 *vs* 82 ± 34, t = 4.36, $p < 0.01$, Fig. 8).

Larval development

On *B. cinerea* cultured on potato-dextrose agar: The mortality rate of the larvae of *E. postvittana* fed on *B. cinerea* on PDA (y = 120.6-120.4*0.89^x) was significantly (p = 0.039) lesser than the larvae that were fed only on PDA (y = 106.61-107.57*0.78^x, where x represented time (d) and y represented the mortality rate (Fig. 9). All larvae were dead at conclusion (maximum15 d).

On *B. cinerea* cultured on Murashige and Skoog medium: The mortality rate of the larvae of *E. postvittana* fed on *B. cinerea* cultured (y = 119.6-

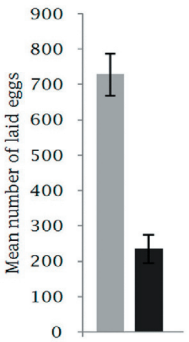

Fig. 6: Mean number of eggs laid by *E. postvittana* in a "free-choice" experiment with uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera.*

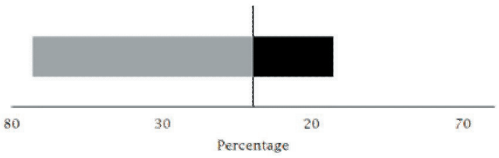

Fig. 7: Response of females *E. postvittana* in a "two-choice" experiment towards uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera.*

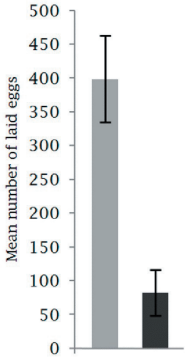

Fig. 8: Mean number of eggs laid by *E. postvittana* in a "two-choice" experiment with uninfected (control) leaves (■) and *B. cinerea*-infected leaves (■) of *V. vinifera.*

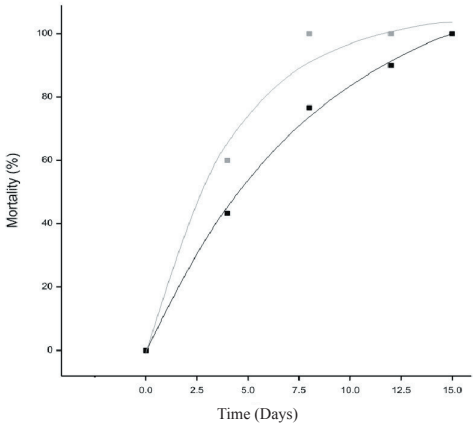

Fig. 9: Mortality rate of *E. postvittana* larvae reared solely on *B. cinerea* (■) and control (or PDA, ■).

119.5*0.87^*x*) was significantly ($p = 0.027$) fewer than the larvae fed on only M-S medium (y = 102.69-102.94*0.71^x, where x = d and y = mortality rate (Fig. 10). All larvae were dead at conclusion (15 d).

On *B. cinerea*-infected and uninfected leaves of *V. vinifera* maintained on Knop's solution: Larvae reared on *B. cinerea*-infected leaves developed faster than larvae reared on uninfected leaves of *V. vinifera* (Tab. 1). The mortality rate of larvae fed on *B. cinerea*-infected leaves (y = 29.68-30.55*0.92^x) was not significantly different than those reared on uninfected leaves of *V. vinifera* (y = 19.65-19.65*0.86^x), where x = d and y = mortality rate (Fig. 11). Pupal masses of female moths reared on *B. cinerea*-infected leaves of *V. vinifera* were significantly greater ($p < 0.05$) than larvae reared on uninfected leaves (Tab. 2). *Botrytis cinerea* infection did not affect pupation and adult emergence percentage,

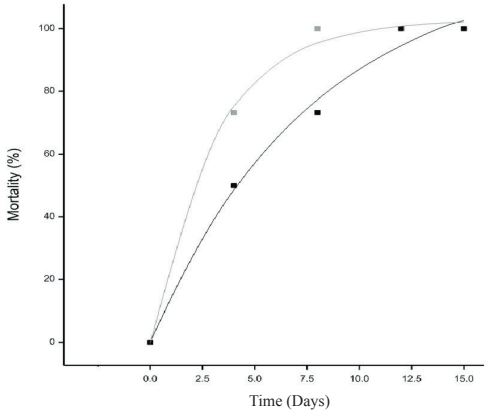

Fig. 10: Mortality rate of larvae reared on *B. cinerea* (■) and control (or M-S medium, ■).

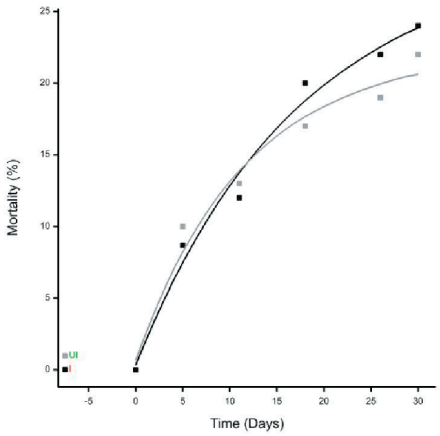

Fig. 11: Mortality rate of larvae reared on *B. cinerea*-leaves (■) or uninfected (■) leaves of *V. vinifera* using Knop's solution.

sex ratio of adult and the length of pre-oviposition period (Tab. 3). *Botrytis cinerea*-infected diet significantly increased the fecundity rates ($p < 0.05$) and viability of eggs ($p < 0.001$) compared with uninfected leaves (Tab. 3).

We found similar effects on larval development and adult performance of *E. postvittana*, when raised on uninfected or *B. cinerea*-infected leaves of *V. vinifera* using PDA and M-S media.

Discussion

Gravid *E. postvittana* prefer to oviposit on uninfected leaves as against that of *B. cinerea*-infected leaves of *V. vinifera*. The larvae of *E. postvittana* which feed on *B. cinerea*-infected leaves develop quicker, attain a heavier pupal mass particularly in those developing as females, and the adults lay more numbers of eggs than those raised on uninfected leaves of *V. vinifera*. The viability rate of eggs hatching to neonates too is greater in those adults reared on *B. cinerea*-infected leaves.

Females of *E. postvittana* do not prefer ovipositing on *B. cinerea*-infected leaves of *V. vinifera* : In the present study, the gravid individuals of *E. postvittana* were deterred during oviposition by the olfactory cues arising from *V. vinifera*

Table 1

Larval developmental time, pupal duration and adult life span (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using Knop's solution

Treatment	Larval developmental time (d)		Pupal duration (d)		Adult life span (d)	
	Male	Female	Male	Female	Male	Female
Uninfelcted leaves	32.5 ± 0.9	34.0 ± 0.8	10.2 ± 0.3	9.3 ± 0.1	8.2 ± 0.7	8.8 ± 0.9
B. cinerea infected leaves	27.9 ± 0.5	30.9 ± 0.5	10.0 ± 0.2	9.2 ± 0.1	7.5 ± 0.9	9.6 ± 0.7
Statistic	t = 2.0	t = 2.8	t = 0.6	t = 1.7	t = 1.9	t = 0.4
p	0.047	0.007	0.50	0.89	0.06	0.69

t = Independent sample t-test.

Table 2

Pupal mass, percentage of larvae surviving from hating to pupae, percentage of larvae surviving from hating to adult and female sex ratio (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected or uninfected leaves of *V. vinifera* using Knop's solution

Treatment	Pupal mass (mg)		% surviving from hatching to pupa	% surviving from hatching to adult	Female sex ratio (%)
	Male	Female			
Uninfelcted leaves	20.9 ± 0.7	27.5 ± 1.1	78	66	46
B. cinerea infected leaves	19.7 ± 0.7	30.6 ± 0.9	76	71	48
Statistic	t = 1.1	t = 2.1	$\chi^2 = 0.11$	$\chi^2 = 0.58$	$\chi^2 = 0.08$
p	0.25	0.04	0.737	0.44	0.77

t = Independent sample t-test.
χ^2 = Contingency table (χ^2).

Table 3

Adult performance (mean ± s.e) of *E. postvittana* reared on *B. cinerea*-infected and uninfected leaves of *V. vinifera* using Knop's solution

Treatment	Delay in egg laying (d)	Fecundity (no. of eggs per female)	No. of larvae emerged per female
Uninfelcted leaves	2.3 ± 0.1	80.0 ± 10.9	67.2 ± 5.8
B. cinerea infected leaves	2.1 ± 0.1	115.1 ± 13.2	106.7 ± 12.3
Statistic	t = 0.98	t = 2.5	t = 2.98
p	0.86	0.01	0.006

t = Independent sample t-test.

leaves infected by *B. cinerea*. In free-choice experiment of this study, when the gravid females of *E. postvittana* had to choose sites for oviposition among *B. cinerea*-infected and uninfected leaves of *V. vinifera*, thus mimicking the natural condition, they deposited significantly more number of eggs on uninfected leaves compared with *B. cinerea*-infected leaves. This observation concurs with our previous findings that individuals of gravid *E. postvittana* are deterred from oviposition when offered *B. cinerea*-infected berries of *V. vinifera* (Rizvi *et al.* 2014 b). Preference for oviposition in different species of Lepidoptera has been shown to be regulated generally by either stimulatory or inhibitory cues arising from host plants (Renwick 1989). Fungus infection can modify the volatiles arising from a plant by interfering with the plant's biosynthetic pathways. Volatiles, arising during pathogenic interactions between plants and fungi, act either as attractants (Cossé *et al*. 1994, Cardoza *et al*. 2003) or deterrents (Dötterl *et al*. 2009, Tasin *et al*. 2011) for insects in three-way, three-component interacting systems. Infection by *B. cinerea* on *V. vinifera* alters its volatile complexion either by changing their quantity or by producing new compounds, such as alcohols, terpenes, and benzoates (Tasin *et al*. 2012). *Botrytis cinerea*-infected berries of *V. vinifera* include several new alcohols (Tasin *et al.* 2012), which deter *L. botrana*. Receptors on the dendritic membrane of sensory neurons of *E. postvittana* and their specific receptivity reiterate that *E. postvittana* is capable of recognizing a range of volatiles (e.g. alcohols, terpenoids and benzoates) and volatile-stress signals such as methyl salicylate (Jordan *et al*. 2009).

A gravid Lepidoptera usually prefers, for oviposition, those sites which maximize its opportunities for a better performance of her progeny (Jaenike 1978). This builds on the premise that juvenile-life stages have a limited capacity

to move on host surfaces and therefore the gravid female chooses the best possible site for oviposition guaranteeing reasonable nutrition to her offspring. In contrast, several arguments and explanations prevail indicating that adult nutrition influences oviposition decision (Mayhew 1997, Scheirs and De Bruyn 2002). For example, *Chromatomyia nigra* (Diptera: Agromyzidae) and *Altica carduorum* (Coleoptera: Chrysomelidae) have been demonstrated to select plants for oviposition that maximize their own fitness rather than that of their progeny, indicating that optimum foraging determines oviposition decisions (Scheirs *et al*. 2000, Scheirs and De Bruyn 2002). In *E. postvittana*, the oviposition preference is essentially governed by plant-surface cues, since it does not lay eggs on rough and hairy surfaces (Tomkins *et al*. 1991, Foster and Howard 1999). Incidence of fungal mycelia on the leaf surfaces of *V. vinifera*, mimicking hairiness precludes oviposition (Rizvi *et al*. 2015, b). The lepidopteran larvae are highly mobile and voracious feeders and, therefore, do not connect strongly with Jaenike's (1978) hypothesis that explains mother's choice of site for oviposition benefitting the offspring (Roitberg and Mangel 1993, Suckling and Brockerhoff 2010). Under experimental conditions, the larvae of *E. postvittana* have been established as highly active feeders on plants of unrelated and various taxa and gravid females lay eggs on those range of plants (Foster and Howard 1999).

Botrytis cinerea-infected leaves positively influence larval performance of *E. postvittana*: Insect and fungus association ranges from non-specific polyphagy to obligate mutualism (Jonsell and Nordlander 2004, Yousuf *et al*. 2014). In the present study, oviposition choices made by adults of *E. postvittana* do not synchronize with the best performance of the larvae. This mismatch could be because either the larvae and adults of *E. postvittana* have differing host requirements or these life forms feed on different organs of different plants. The larvae of *E. postvittana* feed on leaves and fruits of *V. vinifera*, *M. domestica*, and different species of *Citrus*; whereas their adults feed on floral nectar. Our results suggest a mutualistic relationship between the larvae of *E. postvittana* and *B. cinerea.* The larvae develop more rapidly, attain greater pupal mass (particularly in the females), and lay more eggs when fed on *B. cinerea*-infected leaves. Larval diet plays an important role in determining the size and reproductive performance in the Lepidoptera (Mondy and Corio-Costet 2000), where the adult females utilize the energy stored by the larvae for egg production (Boggs 1997). *Botrytis cinerea* plays an essential role in the larval diet and the larvae of *L. botrana* have been demonstrated to disperse the conidia of *B. cinerea* to adjacent berries of *V. vinifera* (Fermaud and Le Menn 1992). Further, the injury caused by the larvae of *L. botrana* while feeding on *V. vinifera* tissues facilitates the colonization and establishment of *B. cinerea* (Fermaud and Lemenn 1992). Cardoza *et al.* (2002) found that leaves of *A. hypogaea* infected with a necrotrophic fungus *S. rolfsii* preferably fed by *S. exigua* and had a positive effect on key life-history traits when fed on *S. rolfsii*-infected *A. hypogaea*.

Botrytis cinerea is an opportunistic necrotrophic fungus, which, under favourable conditions can kill its hosts. The level and nature of infection by fungi can influence the oviposition behaviour of insects (Biere and Tack 2013). It is, therefore, possible that over time, the effect of *B. cinerea* can alter the behaviour of *E. postvittana.* Nonetheless, the caveat here is that the levels of fungal infection used in the present study have indeed been low inducing a sublethal infection on *V. vinifera* leaves. In such a context, the choice of gravid females in avoiding *B. cinerea*-infected leaves of *V. vinifera* could be seen as an adaptive strategy because the difference between the time of oviposition and that of egg hatch, which potentially enhances the infection levels on *V. vinifera* leaves. Therefore, the larval-food quality could have deteriorated with accelerated level of infection or the production of a defensive compound could have reached up to the lethal level (Tasin 2012). This could explain why *B. cinerea*-infected leaves are unattractive for the gravid females for oviposition.

The larvae of *E. postvittana* cannot survive and develop by feeding only on *B. cinerea*: Lepidopteran larvae require high nutritional levels to match their rapid growth before pupation (Slansky and Scriber 1985). For example, the host-plant quality has been shown to have a significant, indirect but positive effect on pupation in *Manduca sexta* (Lepidoptera: Sphingidae) through accelerated growth during its larval stages (Diamond and Kingsolver 2011). Physiological changes in the larvae in preparation for pupation indicate that later instars switch from protein-based diets to lipid-based diets to accumulate more of membrane-bound energy (Stockhoff 1993, Ojeda-Avila *et al*. 2003). Insects are generally shown to lack the capacity to synthesize sterols that are necessary as precursors to steroids which act as growth regulators. The insects, therefore, acquire sterols from their host plants and/or from associated symbionts (Nes *et al*. 1997, Behmer and Nes 2003). Fungi are an excellent source of sterols and vitamins, which are vital in insect development (Mondy and Corio-Costet 2000). Lipids, as principal sources of stored energy and as precursors of steroids are key for insects particularly during metamorphosis (Arrese and Soulages 2010). Nevertheless, high levels of lipids and low levels of other essential nutrients appear to stress insects by affecting metabolism and inducing an inability to maintain homeostasis (Lord 2010). One possible reason for the death of larvae reared on exclusive fungus diet in our study could be that the larvae of *E. postvittana* did not get sufficient energy-containing nutrients, such as carbohydrates and proteins from *B. cinerea*, although they may have had a rich supply of lipids from *B. cinerea*. A second possibility is that the concentrated dose of lipidic materials may have induced a dietary imbalance as shown in the larvae of *M. sexta* parasitized by *Cotesia congregata* (Hymenoptera: Braconidae) (Thompson and Radak 2010).

Conclusion

Gravid females of *E. postvittana* use olfactory and volatile cues to "evaluate" infection levels on *V. vinifera* caused by *B. cinerea* to oviposit. Larvae of *E. postvittana* show a mutualistic relationship with *B. cinerea.* On *B. ci-*

nerea-infected *V. vinifera* leaves, the larvae have a shorter larval duration, attain heavier pupal mass and the emergent adults lay more eggs than the larvae fed on uninfected *V. vinifera* leaves. The larvae reared on exclusive-fungus diet died in maximum 15 d indicating that for a better larval performance and oviposition rate of *E. postvittana*, the *V. vinifera-B. cinerea* interacting system is but imperative although further chemical-ecological verifications are necessary for confirming this.

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