International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 12, Issue: 03 "May-June 2026"

DROSOPHILA MELANOGASTER SELECTED MUTANTS' RESPONSES TO ENTOMOPATHOGENIC FUNGI *METARHIZIUM ANISOPLIAE* STRAIN ME1

*Paschalis Giannoulis

Department of Agriculture, Faculty of Agricultural Sciences,
University of Patras, New Buildings, 30200 Missolonghi, Greece.

*Corresponding Author

DOI: https://doi.org/10.51193/IJAER.2026.12307

Received: 25 April 2026 / Accepted: 12 May 2026 / Published: 25 May 2026

ABSTRACT

Host-response variability to *M. anisopliae* was examined across *D. melanogaster* genotypes differing in immune signaling reporters and cuticular melanization, with particular focus on the yellow (y^1; melanin-deficient) and ebony (e^{11}; hypermelanic) mutants alongside a *GFP-drs/lacZ-dpt* reporter mutant. Flies were reared under controlled conditions (25°C, 12:12 h photoperiod) and challenged with *M. anisopliae* strain ME1. Third-instar larval bioassays used defined inocula (2×10^6 - 2×10^8 sp/ml) and multiple exposure modes and responses were quantified as macroscopic melanization, escape from infection (absence of melanic patches) and pupation timing; developmental suppression assays monitored oviposition, larval emergence, pupation, and eclosion. Both wild type and the *GFP-drs/lacZ-dpt* mutant showed sigmoidal, dose-dependent melanization, with higher inocula reducing latency in melanic patch emergence; genotype effects were modest, supporting the *GFP-drs/lacZ-dpt* mutant as a useful monitoring tool. In contrast, y^1 exhibited strong dose-dependent suppression across development and increased susceptibility, consistent with compromised melanin-associated barrier protection, whereas e^{11} showed rapid full-body melanization and comparatively greater resistance despite clear pathogenic load effects at 10^8 spores/ml.

Keywords: *Drosophila* mutants; Microbial insecticides; Fungal Pathogenicity; Host responses

1. INTRODUCTION

Drosophila melanogaster mutants have proven instrumental in elucidating immune mechanisms with significant implications for understanding agricultural pest immunity. Genetic variants

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affecting critical immune pathways, including the *Toll* and *Imd* signaling cascades as well as cellular processes such as melanization and phagocytosis, exhibit largely independent yet diverse phenotypic responses when challenged with pathogenic organisms, including fungi and bacteria (Ryckebusch et al., 2025). These distinct mutant lines provide valuable model systems for dissecting the complex, multi-layered nature of innate immune responses and their evolutionary conservation across insect species. Upon fungal challenge, the Toll pathway constitutes the primary mediator of antifungal defense responses. Mutants harboring defective or absent functional components of the *Toll* signaling cascade demonstrate markedly increased susceptibility to fungal pathogens, thereby underscoring the pathway's pivotal role in orchestrating effective host defense mechanisms against mycotic infections (Shahrestani et al., 2018). Mechanistic investigations employing genetic mutants have elucidated the critical role of hemocytes in fungal pathogen clearance. These cellular immune effectors function synergistically with humoral defense components including antimicrobial peptides, reactive oxygen species and melanization cascades predominantly derived from the fat body, to orchestrate a comprehensive innate immune response (Lemaitre & Hoffmann, 2007).

Loss-of-function mutations in the X-linked yellow (*y*) gene result in the transformation of the normally dark-pigmented fruit fly to a characteristically pale coloration. The y^1 allele holds particular historical significance as one of the earliest *Drosophila* mutants described and represents the first single-gene behavioral mutant ever characterized in this model organism (Bastock, 1956; Cobb, 2007; Massey, Chung, et al., 2019). Progressive genetic investigations have demonstrated that the yellow gene regulates a complex network of biological functions encompassing cuticular pigmentation, reproductive behavior, and melanin-mediated immune responses (Gompel et al., 2005; Prud'homme et al., 2006). The y^1 allele represents a classical loss-of-function mutation, characterized by an early coding-region alteration that completely abolishes full-length yellow protein production, resulting in a complete pigmentation-null phenotype. The *yellow* protein is secreted, glycosylated, and bears an N-terminal signal peptide (Drapeau, 2003). Insect cuticle coloration arises from a branched biosynthetic pathway that converts tyrosine through sequential intermediates including 3,4-dihydroxyphenylalanine (DOPA) and dopamine, ultimately generating pigment compounds that confer cuticular hues ranging from deep melanization to complete transparency (True, 2003; Wright, 1987).

The ebony (*e*) gene in *D. melanogaster* encodes N-β-alanyl-biogenic amine synthetase, an enzyme that catalyzes the conjugation of β-alanine to both dopamine and histamine (Hartwig et al., 2014). This enzymatic activity plays a crucial regulatory role in cuticle tanning and melanization process, while simultaneously modulating innate immune response (Massey et al., 2019). The classical reference e^{11} mutation produce a dark adult cuticle due to melanin excessive production (Newby & Jackson, 1991). The ebony mutation is also among the earliest catalogued mutants in

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Drosophila, with darkened spiracle sheaths in larvae, lightly colored puparia and a deep brown-black adult (Brehme, 1941). The e^{11} allele, isolated in 1925 is widely used for understanding in insect physiology; e^{11} homozygotes show intense black body pigmentation, lightly colored puparia, disorganized locomotor rhythms in constant darkness, and altered brain biogenic amine levels (Newby & Jackson, 1991; Pantalia et al., 2023; Phillips et al., 2005).

Signaling mutants, particularly the *Drosophila Drosomycin*-GFP reporter line, exhibited *drosomycin* induction following septic injury while simultaneously displaying unexpected constitutive or locally inducible expression patterns in surface epithelia, including the trachea, salivary glands, labellar glands, spiracles, male ejaculatory duct, female spermathecae and seminal receptacle. This epithelial expression occurred independently of Toll pathway activation during immune challenge, suggesting alternative regulatory mechanisms governing *drosomycin* expression in these tissues (Ferrandon et al., 1998).

The utilization of *D. melanogaster* mutants in experimental trials involving fungal entomopathogens can yielded significant insights into the complex interplay between host physiology, immune responses, and pathogen virulence mechanisms. These mutant-based investigations serve as powerful tools for dissecting the immune pathways underlying fungal defense strategies while simultaneously elucidating the physiological consequences of infection. Such research advances fundamental understanding of insect-pathogen interactions, knowledge that proves essential for developing effective biological pest control strategies. Substantial benefits across multiple sectors, from farmers to crop consultants, and research bioscientists are apparent.

The interaction between *D. melanogaster* and the entomopathogenic fungus *Metarhizium anisopliae* represents a paradigmatic model for understanding insect-fungal pathogen dynamics. Upon contact with the host cuticle, fungal conidia undergo rapid germination and develop appressoria generating mechanical pressure and simultaneously secreting cuticle-degrading enzymes, including proteases, chitinases and lipases (Al Abdallah et al., 2026; Beys Da Silva et al., 2010). Pr1 protease activity serves as a biochemical marker of fungal virulence and destruxins disrupt insect host cellular processes suppressing immune responses (Kershaw et al., 1999; Kubicek & Druzhinina, 2007; Paterson et al., 1994)

The role of Baramicin in protecting *Drosophila* against *Metarhizium* species represents an important expansion of our understanding of Toll pathway signaling, which has been predominantly focused on drosomycin (Hanson et al., 2021; Huang et al., 2023). Host-induced antimicrobial peptide provide protection against the toxic effects of destruxin A in glial cells, revealing connections between the nervous system and innate immunity (Huang et al., 2023).

Highly pathogenic *M. anisopliae* isolates can attract target pest species, creating opportunities for 'lure-and-kill' biocontrol strategies. Research with *D. suzukii* has revealed that *M. anisopliae*

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isolates produce volatile compounds that actively attract adult flies, combining behavioral manipulation with direct pathogenesis (Farid et al., 2026).

Herein, host response heterogeneity across genetically distinct *D. melanogaster* lines challenged with the entomopathogenic fungus *Metarhizium anisopliae* strain ME1 was characterized by examining four genotypes: the melanin-deficient y^1 mutant, the hypermelanic e^{11} mutant, the immune reporter *GFP-drs/lacZ-dpt* transgenic line, and wild-type controls. This design enabled examination of the interplay between genetic determinants of cuticular melanin-related pigmentation and fungal resistance. The analysis assessed differential host resistance phenotypes and the relationships between immune function and fungal susceptibility, advancing understanding of genetic factors governing insect-fungus interactions relevant to development of entomopathogen-based biological pest control strategies.

2. METHODS

Insects

Wild type *D. melanogaster* and commercially available stocks were maintained in standard medium culture vials at 25°C under a 12:12h photoperiod. Larval medium included oat flakes (144 g), agar (10 g), black treacle (70 g), nipagin solution (12 ml) per approximately 1 liter. Agar was dissolved in 800 ml of distilled water by heating in a microwave at 93 °C for 3 minutes. Concurrently, oat flakes were soaked in 400 ml of distilled water. Black treacle (70 g) was liquefied by microwaving for approximately 45 seconds, then stirred thoroughly into the oat flake suspension to achieve a homogeneous color. Nipagin solution was added cautiously to the dissolved agar. The agar was subsequently combined with the oat flake mixture and stirred well to ensure uniform dispersion. The complete mixture was then boiled at 93 °C for 10 minutes with continuous stirring. Sterile papers were added after filling the culture vials with medium. Yeast introduced into the vials before the addition of adult flies. For population expansion, breeding groups of 10 females and 4 males were allowed to oviposit for 2 days before sequential transfer to fresh vials.

The stocks were subcultured every 8-10 days (Table 1). The mutant stocks were ordered from Bloomington *Drosophila* Stock Center, Biology Department at Indiana University, US (https://bdsc.indiana.edu/). The immune signaling mutant was kindly provided by Dr. J.M. Reichart [National Centre for Scientific Research (CNRS) in Strasbourg, France].

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Table 1: *D. melanogaster* mutants used in this work

Mutant	*Description*	*References*
Yellow (y^1)	The *yellow* gene (y; FBgn0004034) was among the first sex-linked recessive mutants characterized. Flies hemi- or homozygous for the recessive y^1 allele display a yellow-tan body, bristles, and wings, reflecting the requirement of the yellow protein for converting dopa- and dopamine-derived precursors into black eumelanin. The mutation is pleiotropic: y^1 males also exhibit marked courtship and mating deficits, situating yellow downstream of fruitless in the regulation of sexual behaviour.	Drapeau et al., 2003; Wittkopp et al., 2002; Wittkopp & Beldade, 2009
Ebony (e^{11})	The *ebony* locus (e; FBgn0000527) encodes N-β-alanyl-dopamine (NBAD) synthase, which conjugates β-alanine to dopamine to form the yellow/tan NBAD-sclerotin precursor of wild-type cuticle. The recessive loss-of-function allele e^{11} abolishes enzyme activity, shunting dopamine into the melanin branch and producing a uniformly near-black adult body. Glial ebony activity affects histaminergic visual transmission and circadian locomotor rhythmicity.	Hovemann et al., 1998; Richardt et al., 2003; Suh & Jackson, 2007
Signaling mutant GFP GFP-drs / lacZ-dpt (Drs/lac-z Dipt)	Established reporter genes transgenic line is of innate-immune signaling in *Drosophila*. In *Drs-GFP* flies, GFP placed under the *Drosomycin* promoter reports Toll-pathway activation by fungi and Gram$^+$ bacteria, yielding fat-body fluorescence upon systemic infection alongside local induction in surface epithelia. *Dpt-lacZ* flies, in which β-galactosidase is driven by the *Diptericin* promoter, report *Imd*-pathway activation by Gram-negative bacteria. Together, the two constructs allow clear spatial and temporal discrimination of *Toll* versus *Imd* activity up on a wide range of microbial challenges.	Ferrandon et al., 1998; Lemaitre et al., 1996; Lemaitre & Hoffmann, 2007; Tzou et al., 2000
Wild-Type	Reference strain of *D. melanogaster* carrying wild-type genome and thus providing a baseline for cuticle pigmentation and intact signaling immune response.	Adams et al., 2000; Ashburner et al., 2005; Colomb & Brembs, 2015;

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		Lindsley & Zimm, 1992

Anesthesia of flies

Diethyl ether / carbon dioxide was used. In selection experiments diethyl ether was used because carbon dioxide retarded mating in recovered flies. Carbon dioxide was used for stock culture purposes only.

Entomopathogenic fungus

M. anisopliae strain ME1, originally isolated from pecan weevil, was subcultured from stock cultures on Sabouraud dextrose agar (SDA). Sealed plates were incubated in darkness at 28°C for 2 weeks to promote conidial sporulation, then stored at 4°C for 1 month before use. Spore suspensions were prepared by aseptically harvesting spores from SDA plates using a sterilized bacteriological loop and transferring them to 5 ml of sterile 0.05% Tween 80 solution in centrifuge tubes. Following thorough vortexing and centrifugation at 3,500 rpm for 10 minutes, the pellet was resuspended in fresh 0.05% Tween 80, and spore concentration was determined using a Neubauer hemocytometer. Spore viability was confirmed in vitro by assessing germination rates in bacteriological broth with lactophenol cotton blue staining, achieving initial germination within 4-6 hours and 99% germination within 12 hours.

Larval suppression in feeding vials bioassay

Adult flies (10♀:4♂) were maintained in oviposition vials for 2 days (25°C, 12L:12D) before removal. Resulting progeny were inoculated with 100 µL M. anisopliae spore suspension, and after 3-5 minutes, sterile filter paper strips (1.5 × 3.5 cm) were inserted to enhance pupation surface area and absorb excess solution.

Larval bioassay

Adult flies (10♀:4♂) were placed in fresh vials and allowed to oviposit for 48 h, after which the adults were removed (Figure 1). Vials were maintained at 25 °C under a 12 h light : 12 h dark photoperiod until progeny reached the third larval instar. Larvae were then harvested by flooding each vial with sterile water and agitating gently to dislodge them from the medium. The resulting suspension was decanted onto sterile filter paper supported by a cotton-wool pad, which absorbed excess fluid while retaining the larvae on the filter surface. Larvae were transferred with a fine sterile paintbrush to a Petri dish of sterile water and washed for 60 s to remove residual food debris. After a second filtration step using fresh sterile filter paper and cotton wool, the cleaned larvae were transferred to a Petri dish containing a defined spore suspension and exposed for 60 seconds.

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Inoculated larvae, together with the inverted Petri dish, were placed into an empty culture vial sealed with a moistened bung to maintain humidity. Post-inoculation vials were incubated at 27–29 °C under a 12 h light : 12 h dark photoperiod.

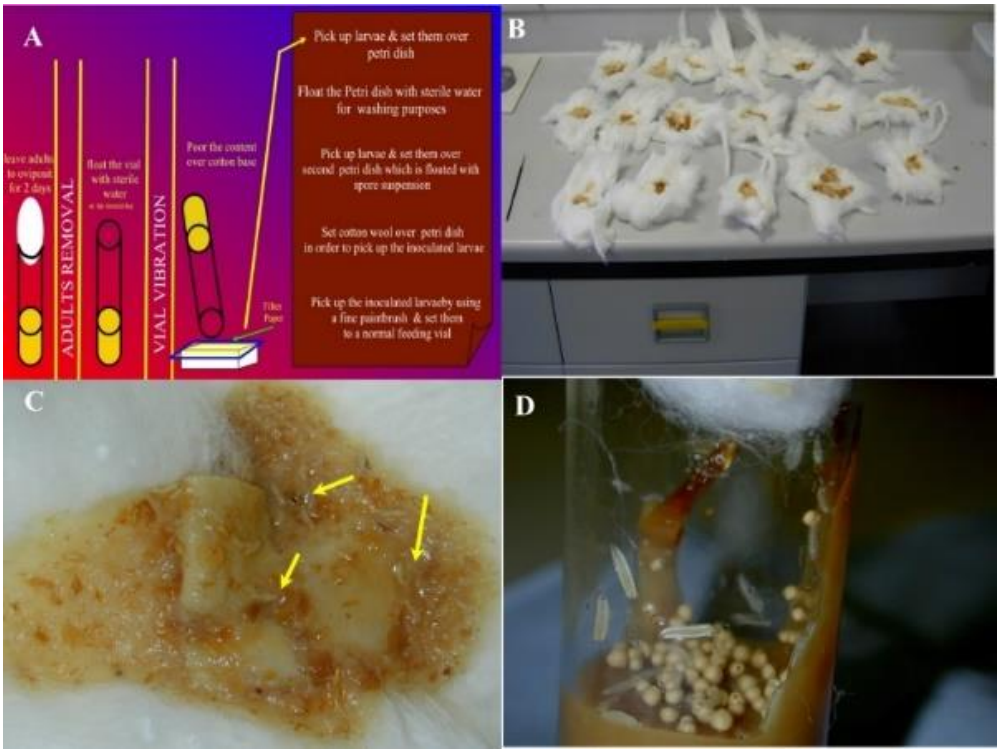

Fig. 1: Graphical representation of the larval bioassay (A), overview of larval extraction process (B), in depth content analysis (C) and post-inoculated larvae set on the feeding vial (D).

Data analysis

Data were analyzed using the 95% confidence limits overlap protocol (Sokal & Rohlf, 2012). The tables and graphical data are presented as mean ± standard deviation of the mean. An α-level of 0.05 was chosen. Prism 8.0 (GraphPad, Boston, MA, USA) was used for data analysis and graph presentation.

3. RESULTS

The effect of *M. anisopliae* strain ME1 on wild type and *GFP-drs / lacZ-dpt* mutant larvae

The responses were monitored following inoculation with three *M. anisopliae* doses (2×10^6, 2×10^7, and 2×10^8 sp/ml), and the cumulative number of responding individuals was plotted against post-inoculation time (Figure 3). Both lines data curves displayed a characteristic sigmoidal profile

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with a clear dose-dependent mode. In the *GFP-drs/lacZ-dpt* reporter line (Figure 2), no melanic patches were detected during the first ten hours at any dose. The highest inoculum (2×10^8) elicited a response from ~13 h, rising sharply to a plateau of approximately 38 individuals by 19–20 h. The intermediate dose (2×10^7) produced a comparable but time-shifted curve, with onset near 14 h and saturation at ~33 individuals by 21 h. The lowest dose (2×10^6) gave a markedly delayed and more gradual response, plateauing at 25 h. A highly comparable pattern was observed in wild type population (Figure 2), although the rising phases were marginally steeper and saturation at the two higher doses was reached slightly earlier, indicating modestly faster response kinetics. Across both panels, the latency to onset was inversely related to inoculum size, while the plateau magnitude scaled positively with dose. The data suggest that the timing and amplitude of the response are robustly governed by the initial fungal load.

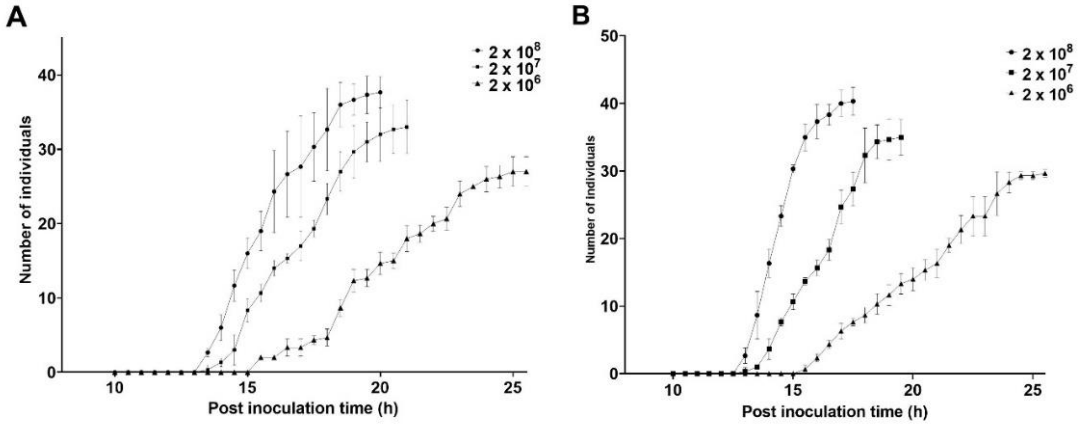

Fig. 2: Emergence of melanic patches on *GFP-drs / lacZ-dpt* mutant (A) and wild type larvae (B) due to *M. anisopliae ME1*

Larval responses to fungal challenge were assessed at three inoculum concentrations (2×10^8, 2×10^7 and 2×10^6 sp/ml) and under three exposure modes, application to the feeding medium, deposition on the vial wall, and placement in an empty vial with a moistened upper bung for both wild-type larvae and the *GFP-drs / lacZ-dpt D. melanogaster* mutant. A clear dose-dependent effect emerged: the number of non-infected larvae increase markedly as inoculum decreased, from 4.75 ± 0.89 to 18.25 ± 2.50 in wild-type individuals exposed on the feeding medium, with a parallel trend in the *GFP-Drs / lacZ-Dpt* mutant (Table 2). Accelerated pupation was most frequent at intermediate to high doses, particularly in the empty-vial condition, suggesting a stress-induced escape response triggered by pathogen contact. Larvae pupating on a normal timescale increased as the inoculum had lower microbial load, mirroring recovery. No substantial differences were detected between the two genotypes in response to *M. anisopliae* on empty vial and when positioned in vial wall, away from feeding medium: wild-type and *GFP-Drs / lacZ-Dpt* larvae

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yielded overlapping means and shared statistical groupings across all treatments, confirming that the reporter constructs did not alter susceptibility or pupation dynamics and validating the use of the transgenic line for any subsequent monitoring of antimicrobial peptide induction.

Table 2: Comparative table on pupation process timing for third instar larval wild type and *GFP-drs / lacZ-dpt* mutant response to *M. anisopliae ME1

Size of inoculum (sp/ml)	Post inoculation positioning	Escaped infection larvae; larvae post-treated with no melanic		Proceed fast to Pupation (1-6h)		Proceed normal to pupation (>24h)	
		Wild Type	*GFP-drs /lac-z dpt*	Wild Type	GFP-drs /lac-z dpt	Wild Type	*GFP-drs /lac-z dpt*
2×10^8	placed on feeding medium	4.75 ± 0.89^a	5.25 ± 1.70^a	3.75 ± 1.67^a	5.00 ± 1.41^a	1.00 ± 0.81^a	0.75 ± 0.82^a
2×10^7		12.00 ± 1.7^{ab}	9.25 ± 3.26^{ab}	5.75 ± 1.88^b	5.75 ± 2.55^{ab}	6.25 ± 2.16^{ab}	3.50 ± 0.94^a
2×10^6		18.25 ± 2.50^b	17.25 ± 2.19^b	7.25 ± 2.50^b	8.00 ± 2.62^b	11.00 ± 1.63^b	9.25 ± 1.71^a
2×10^8	ditto on feeding vial wall	10.25 ± 1.25^a	7.25 ± 2.06^a	8.00 ± 0.81^a	5.75 ± 1.50^a	2.25 ± 0.95^a	1.50 ± 1.00^a
2×10^7		14.25 ± 2.06^a	12.75 ± 2.06^a	7.50 ± 3.87^a	7.00 ± 1.82^a	6.75 ± 2.87^a	5.50 ± 1.73^a
2×10^6		19.75 ± 3.3^a	19.5 ± 4.2^a	8.75 ± 1.70^a	9.25 ± 1.89^a	10.75 ± 4.99^a	10.50 ± 3.10^a
2×10^8	Ditto Empty vial (upper bung moistened)	12.3 ± 2.08^a	9.66 ± 2.08^a	9.33 ± 2.08^a	8.66 ± 2.08^a	3.00 ± 0.00^a	1.00 ± 1.00^a
2×10^7		17.00 ± 3.40^a	15.00 ± 2.64^a	11.60 ± 2.51^a	9.66 ± 2.88^a	4.30 ± 0.57^a	5.33 ± 2.30^a
2×10^6		21.6 ± 2.51^a	20.3 ± 0.57^a	10.60 ± 2.08a	9.33 ± 2.08a	11.00 ± 1.00^a	11.00 ± 1.73^a

**Data represent mean ± standard deviation (n=4, N=50), different letters demonstrate significant differences ($p < 0.005$).*

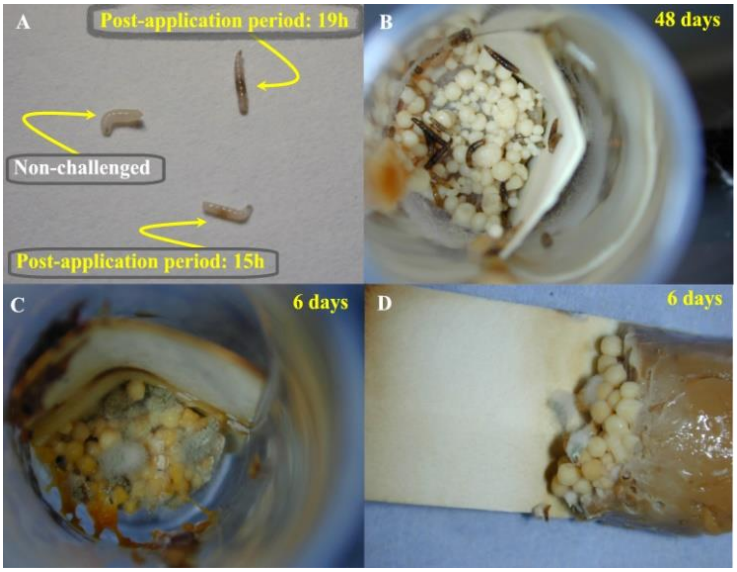

Fig. 3: Melanization of *GFP-drs / lacZ-dpt* mutant after exposure to *M. anisopliae ME1*: Appearance of melanic patches (A), extensive darkening of dead larvae (B), extensive cadaver sporulation on 6 days (C).

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For yellow mutant the growing into vial - *Metarhizium* application bioassay revealed significant treatment effects across all developmental stages (Figure 4). Oviposition rates were highest in non-treated controls (75.2 ± 2.1 individuals per vial), with no significant differences. Larval emergence from medium showed reduced patterns as applicable fungal dose increases, with controls maintaining the highest emergence rates. Spore treatments progressively reduced emergence, with 10^8 sp/ml showing the most pronounced effect. Pupation success was severely compromised across all spore treatments. Similar patterns were observed during eclosion, compared to untreated controls. The data demonstrate dose-dependent effects.

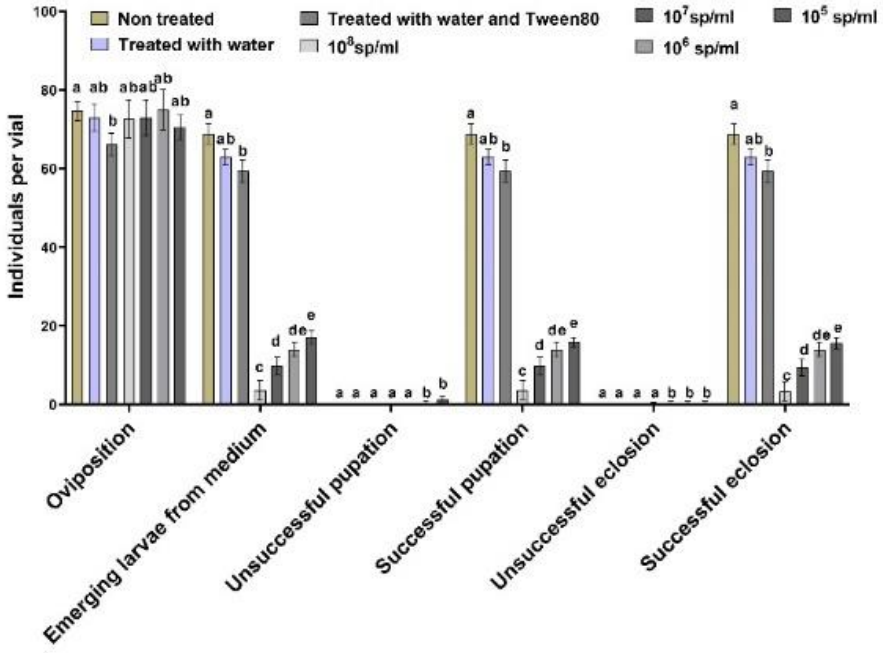

Fig. 4: Effect of *M. anisopliae ME1* on *y1* mutant *D. melanogaster*. Values are means ± SD (n = 5, N = 10). Different letters indicate significant differences (p < 0.005).

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Fig. 5: Extensive melanization of *D. melanogaster* y^1 larval populations in feeding vial, 24 hours following exposure to *M. anisopliae ME1* at 10^8 spores/ml.

For ebony mutant, the bioassay revealed significant treatment effects across all four developmental stages examined (Figure 6). Initial oviposition success ranged from 56.8 ± 2.1 to 72.3 ± 4.7 individuals per vial across all treatments. The highest spore concentration (10^8 sp/ml) and both control treatments (water alone and water + Tween80) showed statistically similar performance, while intermediate concentrations (10^6 and 10^7 sp/ml) demonstrated comparable but slightly elevated oviposition rates. Non-treated controls exhibited the highest oviposition success. A marked reduction in survival was observed during the transition from oviposition to larval emergence. The 10^8 sp/ml treatment group showed the lowest emergence rates, while non-treated and 10^6 sp/ml groups maintained higher survival. Pupation success remained consistent with larval emergence patterns. Treatment effects persisted with similar statistical groupings, indicating sustained impact of the biological agent through developmental stages. Final adult emergence mirrored pupation results, with the highest spore concentration maintaining the most pronounced suppressive effect compared to controls and lower concentrations. A clear dose-dependent response was evident, with the 10^8 sp/ml concentration consistently producing the greatest reduction in developmental success across all stages, confirming the pathogenicity effect based on microbial load.

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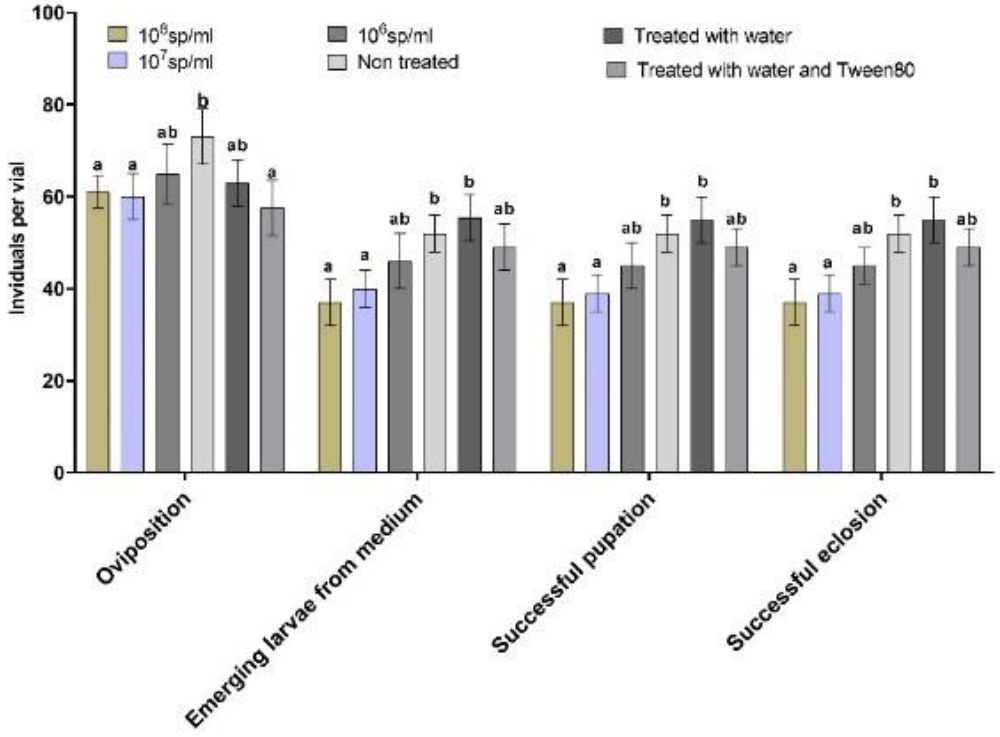

Fig. 6: Effects of *M. anisopliae ME1* on e^{11} mutant *D. melanogaster*. Data are mean ± SD (n = 5, N = 10). Different letters indicate significant differences ($p < 0.005$).

4. DISCUSSION

This investigation examined how genetic variation in cuticular melanization and immune signaling affects *D. melanogaster* susceptibility to the entomopathogenic fungus *M. anisopliae* strain ME1, clarifying the relative contributions of melanin-mediated barrier protection and immune signaling in antifungal defense. The *GFP-drs / lacZ-dpt* transgenic flies exhibited modestly increased susceptibility to *M. anisopliae* infection relative to wild-type line. Visual inspection revealed that these reporter flies appeared consistently smaller than wild-type individuals maintained at equivalent population densities. Melanization patterns following fungal challenge showed distinct temporal differences between genotypes. While melanic patches emerged simultaneously on the ventral cuticle of both transgenic and wild-type flies, the reporter strain required approximately twice as long to progress to complete body melanization. Light microscopy of infected larvae revealed that these melanic patches were predominantly localized to internal tissues proximal to hematopoietic gland. The yellow (y^1) mutant demonstrated enhanced susceptibility to *M. anisopliae* compared to wild-type flies, suggesting that melanin deficiency reduces antifungal resistance in *D. melanogaster*. However, definitive interpretation of this phenotype is constrained by limited genomic characterization. The observed, increased susceptibility cannot be attributed

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unequivocally to reduced melanin production, as pleiotropic effects may contribute to the phenotype. Impaired melanin synthesis in Lepidoptera elevates cuticular β-alanine levels, a metabolic alteration potentially conserved in Drosophila (Koch et al., 2000). Conclusive demonstration that yellow gene function directly modulates fungal susceptibility through melanin-dependent mechanisms would require comparative analysis of additional alleles at this locus.

The resistant phenotype of e^{11} mutants cannot be attributed solely to melanin deposition, but likely involves complex interactions between altered cuticle composition, modified phenoloxidase regulation, and pleiotropic effects on immune gene networks. This complex resistance requires investigating signaling cascades, cuticle modifications, and effector production to understand antifungal immunity.

Future investigations should elucidate differential susceptibility of yellow (y^1) and ebony (e^{11}) *Drosophila* mutants to *M. anisopliae* through complementary approaches. Molecular characterization of cuticle integrity requires ultrastructural and biochemical analyses, including electron microscopy, lipid and hydrocarbon content analysis, to determine how melanin deficiency compromises barrier function. Comparative transcriptomic and proteomic profiling should examine pigmentation-immunity crosstalk, particularly signaling pathway activation and melanization cascades. Quantitative assays measuring reactive oxygen species and antioxidant activities would clarify melanin's role in oxidative stress management. Epigenetic investigations may identify regulatory mechanisms linking pigmentation to immune responses, while extending studies to additional entomopathogens would assess the ecological relevance of these phenotypic differences. Finally, genetic complementation through transgenic expression of yellow and ebony genes in their respective mutants would confirm the causative role of melanin-related pathways in modulating cuticle integrity and immune defense, establishing direct mechanistic links between pigmentation genetics and fungal susceptibility.

5. CONCLUSION

This study examined how genetic variation in cuticular melanization affects *D. melanogaster* susceptibility to *M. anisopliae*. Wild-type and *GFP-drs / lacZ-dpt* flies exhibited host responses with comparable susceptibility patterns. The y^1 mutant demonstrated pronounced developmental suppression and high levels of susceptibility, consistent with compromised cuticle integrity due to melanin absence. Conversely, the e^{11} mutant showed enhanced resistance, despite significant dose-dependent effects at high fungal loads. These findings support a functional linkage between cuticle pigmentation and immunity, however, further investigation at whole genome level is needed.

Ethics Statement

Not applicable: This manuscript does not include human or animal research.

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ACKNOWLEDGEMENTS

Sincere gratitude is expressed to: a) A.K. Charnley from the Department of Biology and Biochemistry, Bath University (UK) for providing valuable suggestions and creating a stimulating environment for this work, and b) J.M. Reichart from the National Centre for Scientific Research (CNRS) in Strasbourg (FR), for providing the *GFP-drs / lacZ-dpt* mutant.

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