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Entomopathogenic fungi in passion fruit pests: A review and preliminary report of *Fusarium* sp. on *Agraulis vanillae*

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Abstract: *Agraulis vanillae* Linnaeus, 1758, and *Dione juno* Cramer, 1779 (Insecta: Lepidoptera: Nymphalidae) are recognized as significant pest insects in passion fruit (*Passiflora edulis* – Passifloraceae) cultivation, causing substantial economic losses to farmers. Despite their agricultural relevance, there is still a noticeable scarcity of scientific records documenting fungal diseases that could affect these species. This lack of information hinders the development of effective and sustainable pest control strategies, which are highly necessary for reducing pesticide dependency. To address this knowledge gap, a comprehensive literature review was conducted to identify evidence and descriptions of infections or mortality caused by fungal pathogens in *A. vanillae* and *D. juno*. As part of this research, larvae of A. vanillae infected with a fungal pathogen belonging to the genus *Fusarium* (Fungi: Hypocreales: Nectriaceae) were collected in Vera Cruz, Rio Grande do Sul, Brazil. The taxonomic characteristics and cultivation conditions of this fungal isolate are thoroughly detailed in this study. This discovery holds significant potential for agricultural applications, offering an alternative to chemical pesticides in the management of passion fruit pests. However, further experimental studies are essential to evaluate the biocontrol efficacy and practical applicability of *Fusarium* sp. under field conditions. This work expands knowledge on fungal pathogens of agricultural pests and emphasizes their role in integrated pest management systems.

Key words: Biocontrol, Fungi, Passion fruit plague, Review.

Fungos entomopatogênicos em pragas do maracujá: uma revisão e relato preliminar de *Fusarium* sp. em *Agraulis vanillae*

Resumo: *Agraulis vanillae* Linnaeus, 1758, e *Dione juno* Cramer, 1779 (Insecta: Lepidoptera: Nymphalidae) são reconhecidos como insetos-praga significativos no cultivo de maracujá (*Passiflora edulis* – Passifloraceae), causando perdas econômicas substanciais aos agricultores. Apesar de sua relevância agrícola, ainda há uma notável escassez de registros científicos que documentam doenças fúngicas capazes de afetar essas espécies. Essa lacuna de informações dificulta o desenvolvimento de estratégias de controle de pragas eficazes e sustentáveis, necessárias para reduzir a dependência de pesticidas químicos. Para preencher essa lacuna de conhecimento, foi realizada uma revisão abrangente da literatura com o objetivo de identificar evidências e descrições de infecções ou mortalidade causadas por patógenos fúngicos em *A. vanillae* e *D. juno*. Como parte desta pesquisa, larvas de *A. vanillae* infectadas com um patógeno fúngico pertencente ao gênero *Fusarium* (Fungi: Hypocreales: Nectriaceae) foram coletadas em Vera Cruz, Rio Grande do Sul, Brasil. Este estudo detalha as características taxonômicas e as condições de cultivo desse isolado fúngico. Essa descoberta apresenta um potencial significativo para aplicações agrícolas, oferecendo uma alternativa aos pesticidas químicos no manejo de pragas do maracujá. No entanto, estudos experimentais adicionais são essenciais para avaliar a eficácia do biocontrole e a aplicabilidade prática de *Fusarium* sp. em condições de campo. Este trabalho amplia o conhecimento sobre patógenos fúngicos de pragas agrícolas e enfatiza seu papel em sistemas integrados de manejo de pragas.

Palavras-chave: Biocontrole, Funga, Pragas do maracujá, Revisão.

1. INTRODUCTION

Passion fruit and passion fruit tree refer to different species within the Passifloraceae family, all belonging to the genus *Passiflora* (FALEIRO et al., 2019). They are herbaceous or woody climbing plants, clinging to supports by tendrils (LUNZ et al., 2006). Passion fruit is a fruit of economic interest, highly nutritious, and with medicinal value, consumed fresh or used in juice production (THOKCHOM & MANDAL, 2017). The most cultivated species is yellow passion fruit (*Passiflora edulis Sims f. flavicarpa* Deg.), with Brazil being the world's largest producer, selling both domestically and internationally (LIMA et al., 2006; FALEIRO et al., 2019). Passion fruit cultivation can be attacked by various insect species; however, two species of defoliating caterpillars: *Dione juno* and *Agraulis vanillae* stand out as pests due to their frequency, especially in dry periods of the year (LIMA et al., 2006; LUNZ et al., 2006; ALMEIDA et al., 2021).

Dione juno Cramer, 1779 (Insecta: Lepidoptera: Nymphalidae) has distribution throughout Central America, much of South America, and the Lesser Antilles (TAVARES et al., 2002). The larvae of this species are yellowish, alternating to dark brown throughout their development. Additionally, their hindwings have brown spots, and in the adult phase, the forewings are orange with black outer margins (MACHADO et al., 2017). Oviposition preferably occurs on the abaxial surface of leaves, with eggs grouped in numbers ranging from 60 to 140 (MACHADO et al., 2017; TAVARES et al., 2002).

Bianchi & Moreira (2005) reported the adaptability of *D. juno* to consume different species of the *Passiflora*. The preference for consuming newly hatched larvae and fifth instar larvae is directed towards the *P. edulis* genotype compared to other passifloraceae genotypes. *D. juno* deserves attention for its incidence throughout the year in passion fruit cultivation and the damage caused due to the larvae's gregarious behavior, leading to increased plant consumption (TAVARES 2002; ANGELINI et al., 2007). In young plants, the damage can be more severe, as larvae can cause total defoliation and scrape the bark of passion fruit tree branches (LIMA et al. 2006).

On the other hand, gulf fritillary, *Agraulis vanillae* Linnaeus, 1758 (Insecta: Lepidoptera: Nymphalidae) is a butterfly species that, during its juvenile phase, is a solitary caterpillar. Its distribution is neotropical, ranging from the southern United States, the West Indies to northern Argentina and Uruguay (SILVA et al., 2006). In the adult phase, it stands out for its orange wings with black spots, while the larval phase at the beginning of its cycle is described with whitish-brown coloration, turning to dark yellow at the end of the cycle with two lateral brown stripes and a body covered in black spines (SILVA et al., 2006; MACHADO et al., 2017).

Species of the genera *Agraulis* are recognized for their association with passion fruit trees (*Passiflora edulis*), and since 1950s, their predation has been cited as harmful to the plant's leaves, reducing its photosynthetic area (LORDELLO, 1954). Studies suggest coevolution between herbivorous butterflies and their target plants: the larvae of the *Heliconiii tribe* (Nymphalidae) are well-adapted to passion fruit trees, showing great specificity between the insect and the plant. For example, *A. vanillae* females deposit their eggs on *Passiflora* species most suitable for larval growth and development (COPP & DAVENPORT, 1978b). Oviposition is guided by chemical 'cues' activated by contact, and the infestation level is related to the balance of compounds presented by *Passiflora* species; thus, the population remaining on the plant depends on the oviposition rate and larval mortality rate (COPP & DAVENPORT, 1978a).

Several methods can control both species: for small crops, manual removal of caterpillar eggs is recommended, while for larger crops, this becomes impractical and inefficient (LUNZ et al., 2006). Pest control can be achieved through the use of pesticides; however, indiscriminate use can lead to ecological imbalance by eliminating not only the target pest species but also other vertebrate and invertebrate organisms (LIMA & VEIGA, 1995). Other consequences may include health problems for the pesticide applicator and, more importantly, the end consumer of the product (ALI et al., 2021). Problems related to pesticide use have stimulated research to discover biological control methods, and currently, the use of bacteria, viruses, and parasitoids is encouraged (LIMA & VEIGA, 1995; MACHADO et al., 2017).

Insects that can act as biological controllers are common in the nymphalid family, among which the genus *Agraulis* includes the predation of caterpillar larvae by *Polistes* sp. (Hymenoptera) and parasitism in pupae by *Spilochalcis* spp. (Hymenoptera) (LIMA & VEIGA, 1995). Currently, the control of *D. juno* and *Agraulis vanillae* uses *Bacillus thuringiensis* var. kurstaki and *Baculovirus dione* (NPV), while other strategies rely on parasitoids like *Trichogramma* (Hymenoptera) (MACHADO et al., 2017).

Entomopathogenic fungi stand out in biological control by indirectly affecting insect behavior, contributing not only to the direct control of insect pests but also to enhancing plant resistance against agronomically significant pests and phytopathogenic microorganisms. Among the most notable examples are *Metarhizium anisopliae* and *Beauveria bassiana*, widely recognized for their efficacy. Furthermore, these fungi exhibit compatibility with predators and parasitoids, without compromising the fitness or behavior of these allies in integrated pest management, suggesting a synergistic potential that warrants further exploration. Looking ahead, their complementary multitrophic attributes, such as promoting plant growth and increasing tolerance to environmental challenges like drought, underscore their relevance. Their ability to persist in the environment by recycling inoculum on insect cadavers contrasts favorably with the persistence of chemical residues, positioning them as a promising and effective alternative for sustainable pest management (MANTZOUKAS et al., 2022a; QUESADA-MORAGA, 2022).

In this context, this work aims to conduct a literature review of fungal pathogens found in *D. juno* and *Agraulis vanillae* and to report a new infectious agent for *A. vanillae*.

2. MATERIAL AND METHODS

2.1. Literature review

Searches were conducted in platforms: Google Scholar (https://scholar.google.com.br), and Web of Science (https://clarivate.com) with the key words: '*Dione juno* and Fungi', '*Dione juno* and Entomopathogenic', '*Agraulis vanillae* and Fungi', '*Agraulis vanillae* and Entomopathogenic'. Articles published between the years 1964 and 2022 were selected, this period was chosen to compile a comprehensive list of entomopathogenic fungi associated with these species. Monographs, dissertations and theses were excluded from the search.

Aiming to conjecture what the main research topics were involving *D. juno* and *A. vanillae*, the VOZviewer 1.6.19 software was used to create a bibliometric map of keywords. The terms "*Agraulis vanillae*" and "*Dione juno*", "*Dione juno* or *Agraulis vanillae*" were searched in Web of Science records (https://clarivate.com). In order to find expressive keywords to create a clearer map, only keywords with at least two occurrences in the articles found were chosen.

2.2. Collection, Fungal Isolation, and Taxonomy

A larva of A*graulis vanillae* was collected under *Passiflora edulis* leaves covered with fungal mycelium in April 2022 in the municipality of Vera Cruz – RS – Brazil, in a residential area (-29.720074, -52.497462). The fungus was isolated from the mycelial growth of the collected larva and cultivated on Sabouraud medium in Petri dishes. The isolate was subcultured every 15 days and kept at 26 °C (± 2°C) without light exposure in the Fungus Taxonomy Laboratory in São Gabriel - RS (UNIPAMPA). For taxonomic identification, slides were prepared from fragments of the isolated culture for visualization under a Zeiss ACT0 optical microscope. The isolate was identified as *Fusarium* sp. following Nelson et al. (1990).

2.3. Confirmation of *Fusarium* sp. entomopathogenicity

Entomopathogenicity tests followed Koch's postulates to confirm *Fusarium* sp. as a disease agent in *Agraulis vanillae* (GENTRY et al., 2021). The steps include isolating the pathogen, infecting new hosts, and re-isolating it (KOCH, 1878). An axenic culture was obtained from a larva collected in Vera Cruz, RS, Brazil (-29.720074, -52.497462). Four healthy larvae from the same site were used: three were placed on the fungal culture in Sabouraud medium Petri dishes for 10 minutes and then transferred to clean Sabouraud medium dishes, while one was kept as a non-inoculated control. The inoculated larvae showed mycelial growth and paralysis over 7 days (Section 3.3), and the fungus was re-isolated on Sabouraud medium at 26 °C (± 2°C) for 15 days, using a modified method by Ramakuwela et al. (2020). No fungal growth was observed in the control. Spore amounts were not quantified, and only four larvae were tested.

3. RESULTS

3.1 Bibliographic Keyword Maps

3.1 Bibliometric Insights into Biological Control Research for *Dione juno* and *Agraulis vanillae*

Bibliometric keyword maps were generated using VOSviewer 1.6.19 to explore the research landscape surrounding *Dione juno* and *Agraulis vanillae*, with a specific aim to assess the emphasis on entomopathogenic fungi as biological control agents for these passion fruit pests. Three maps were produced from Web of Science records: one for "*Agraulis vanillae*" (Figure 1), one for "*Dione juno*" (Figure 2), and a combined map tracking keyword evolution over time (Figure 3). Each map organizes keywords into color-coded thematic clusters based on co-occurrence.

The *A. vanillae* map (Figure 1) revealed four clusters: the red cluster ("butterfly," "diversification," "genetic diversity," "evolution," "nymphalids lepidoptera") reflects taxonomic and evolutionary studies; the yellow cluster ("pyrrolizidine alkaloids," "toxic nectar," "ithomiine butterflies," "pollination") focuses on chemical ecology; the blue cluster ("insects," "genes," "methyl jasmonate," "passion fruit," "herbivory") links to plant-insect interactions; and the green cluster ("*Agraulis vanillae*," "nectar," "migration," "phenology") addresses ecological dynamics. For *D. juno* (Figure 2), four clusters emerged: the red cluster ("*Agraulis vanillae*," "gene," "protein," "baculoviruses") highlights molecular and viral control studies; the blue cluster ("lepidoptera," "identification," "genome sequence") focuses on taxonomy; the yellow cluster ("passion fruit," "*Passiflora edulis*") ties directly to the host plant; and the green cluster ("*Dione juno*," "evolution," "heliconian butterflies," "flavonoids") explores evolutionary and phytochemical relationships. The combined map (Figure 3) illustrates keyword shifts over time for both species.

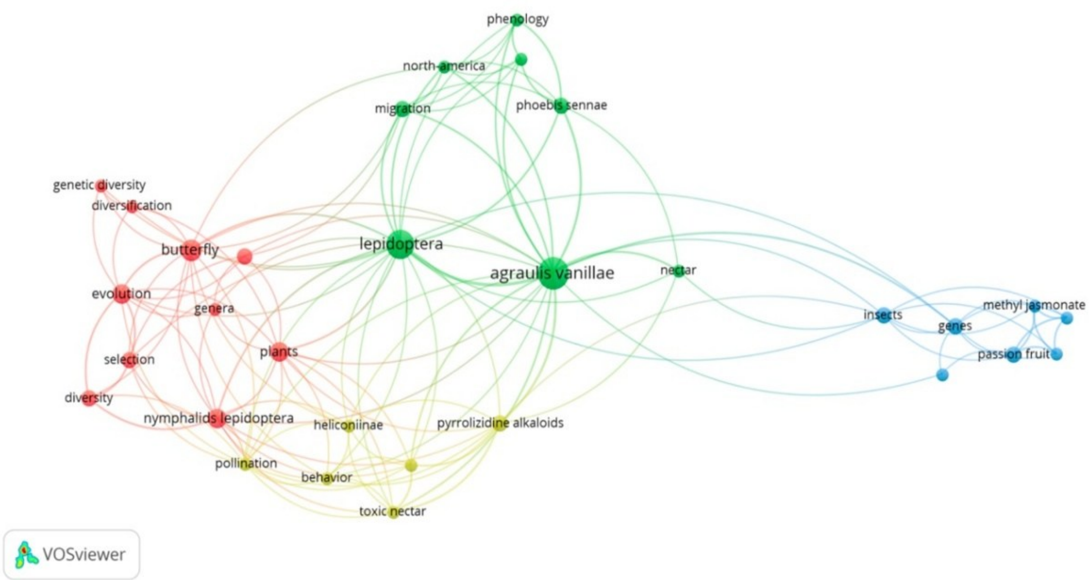

Figure 1. Bibliographic keyword map related to the species '*Agraulis vanillae*.'

Notably, while these maps capture diverse research themes: genetics, ecology, and host-plant dynamics; entomopathogenic fungi were underrepresented. The term "baculoviruses" (Figure 2, red cluster) was the only biological control agent identified, indicating a research gap in fungal-based control for these species. This finding aligns with the scarcity of fungal pathogen records in the literature (Section 4) and underscores the significance of this study's identification of *Fusarium* sp. as a novel entomopathogenic agent for *A. vanillae*, as well as the need for further exploration of fungal control options for *D. juno*.

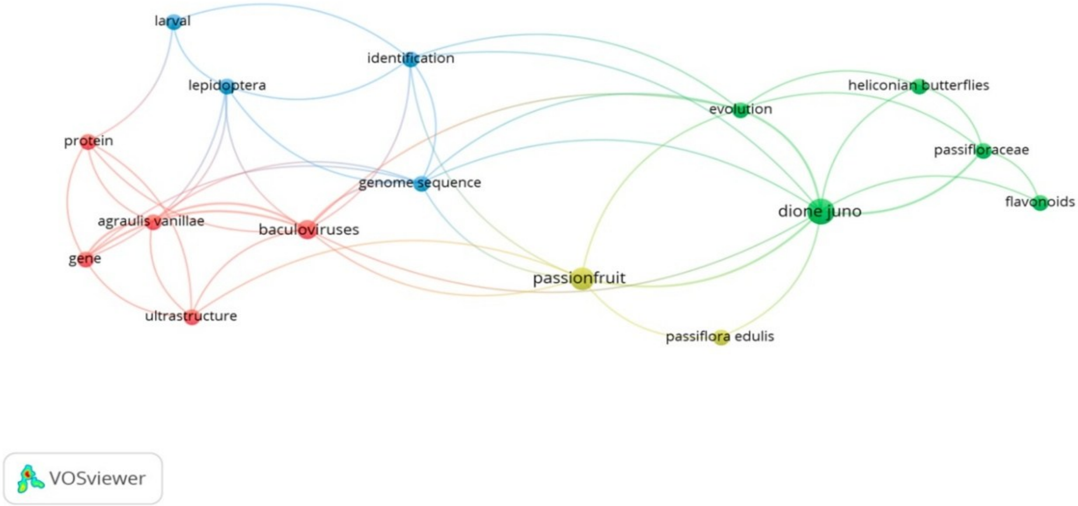

Figure 2. Bibliographic keyword map related to the species '*Dione juno'*.

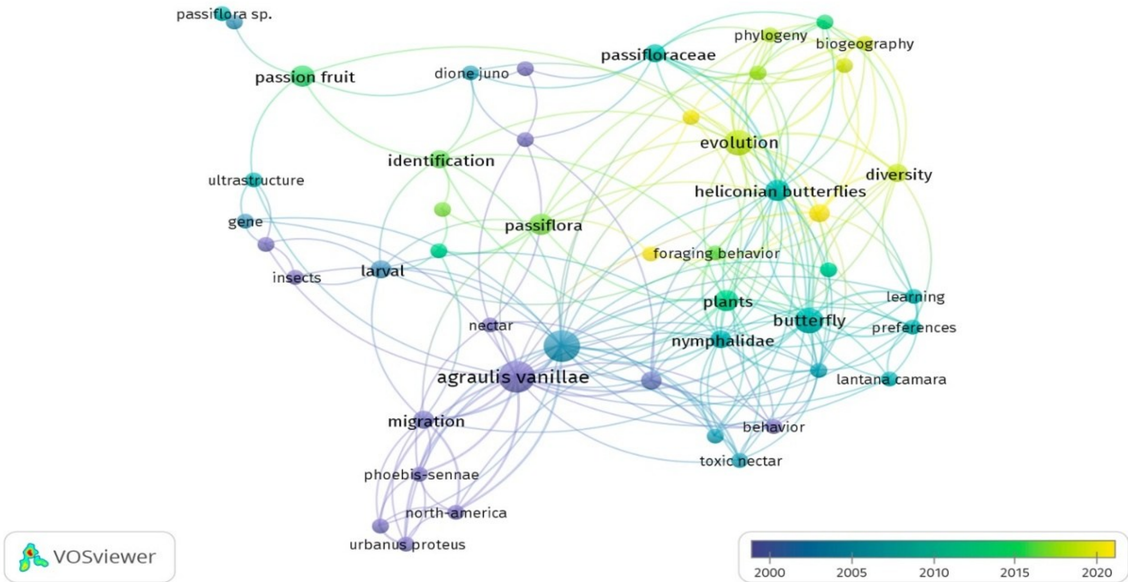

Figure 3. Bibliographic map for '*Agraulis vanillae* or *Dione juno*' and the change of keywords over time.

3.2. Morphological characterization of *Fusarium* sp.

The colonies on *Sabouraud medium* (Figure 4A) exhibited radial growth in 7 days with a range of 3.5cm – 4 cm. The height of the aerial mycelium ranged from 0.5 mm (lower) to 3.4 mm (higher) in 30 days, showing a white color (top view), while the bottom view exhibited a light brown to orange hue. Conidiophores with monophialides and polyphialides produced macroconidia and microconidia. The conidia of the aerial mycelium are characterized by the presence of macroconidia dorsiventrally unmarked, maintaining a straight curvature along the spore, basal cells in a foot-like or toothed shape, relatively thin with thin cell walls, noticeable curvature in the basal cell, septation of 1-7 septa: 1-septate: (10)11.25 - 18(24) X (2) 2 - 2.12(2.5) µm, 2-septate: (16)16 - 23(26) X (2)2 - 3.5(4) µm, 3-septate: (18)20- 28.5(30) X (2)2 - 4(4) µm, 4-septate:(20) 20.5 - 30.5(32) X (2)2 - 4(4) µm, 5-septate: (20)21 - 30.5(36), 6-septate: (22)22.5 - 31.25(33) X (2)2 - 4(4) µm, 7-septate: (22)23 - 30(30) X (2)2 - 4(4) µm. Microconidia were also present and abundant, presenting an oval to reniform clavate shape. Chlamydospores were also present, solitary and scattered.

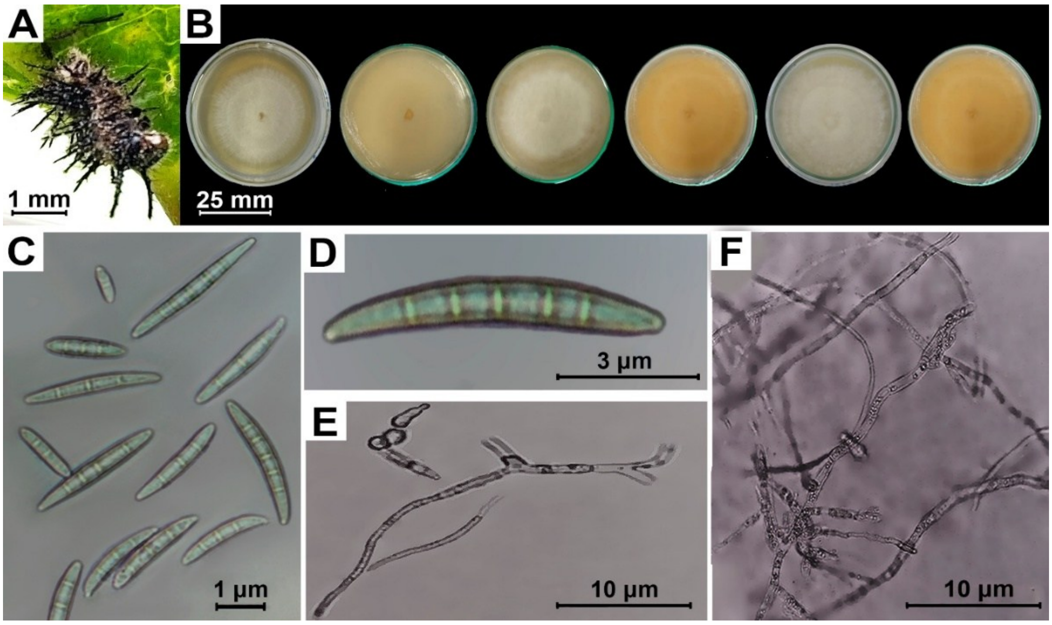

Figura 4. A. Caterpillar of *Agraulis vanillae* affected by the fungal pathogen. B. Top and bottom views of three *Fusarium* sp. isolates grown on *Sabouraud medium* for 30 days, from left to right. C–F. Observed under 100x objective with immersion oil: C. Microconidia and septate macroconidia. D. Macroconidium. E. Chlamydospore. F. Aerial mycelium.

3.3. Test following Koch's postulate

The three *Agraulis vanillae* larvae exposed to Fusarium sp. for 10 minutes on the fungal culture showed paralysis after 4 hours and were fully covered by mycelium within 3 days. The control larva, not inoculated, remained active with no fungal growth over 7 days. Fusarium sp. was re-isolated from the infected larvae, supporting its role as a potential pathogen. Due to the small sample size and simplified method, these results are preliminary, as discussed in Section 4.

4. DISCUSSION

As indicated in Table 1, the oldest record of fungal infections in *Agraulis vanillae* dates back to a description on the island of Cuba, mentioning the fungus *Lecanicillium lecanii* (Zimm.) Zare and W. Gams 2001. The symptoms observed in larvae included loss of appetite, weakness, disorientation, and color loss (ESCANDÓN-ARBOLAY et al., 2013). *L. lecanii* was later reclassified into the genus *Akanthomyces* in 2017, discovered by Nivter in 1861 in present-day Sri Lanka (JIANG & WANG, 2023). It is a commercially widespread pathogen, primarily used in plant greenhouses (JIANG & WANG, 2023). In India, at least 60 products based on *A. lecanii* are registered, in dry or liquid formulations, considered the most efficient mycoinsecticide against aphids (KUMAR et al., 2019).

Table 1. Pathogenic fungi of Agraulis vanillae and Dione juno mentioned in peer-reviewed studies.

Insect	Enthomopathogen	Country	Reference
Agraulis vanillae insularis	*Akanthomyces lecanii* (formely *Lecanicillium lecanii*)	Cuba	Escandón-Arbolay M. C. et al., 2013
Agraulis vanillae	*Cordyceps tenuipes* (formerly *Isaria tenuipes*)	Colômbia	L. A. Castrillo., 2020
Dione juno	*Beauveria bassiana*	Peru	Malpartida-Zevallos, J. et al.., 2013

The latest record is from Colombia, where the pupa of *Agraulis vanillae* was infected with the fungus *Isaria tenuipes* Peck 1878 (CASTRILLO, 2020). It has since been reclassified into the genus *Cordyceps* (JIANG & WANG, 2023). In experiments targeting *Aedes aegypti* larvae, the dengue vector, *C. tenuipes* demonstrated good efficacy, reducing the levels of certain enzymes responsible for pest resistance to chemical toxins, coupled with its non-toxic activity to aquatic predators (KARTHI et al., 2020).

For *Dione juno*, there was only one finding in an experiment testing larval mortality upon contact with *Beauveria bassiana*, a fungus known for its control of lepidopterans and coleopterans (MALPARTIDA-ZEVALLO et al., 2013). The tests identified that third-instar larvae of *Dione juno* were susceptible to *Beauveria bassiana* (Bals.-Criv.) Vuill. 1912, discussing the potential use of *B. bassiana* for integrated pest management in the field (MALPARTIDA-ZEVALLO et al., 2013). *Beauveria bassiana* stands out for the presence of secondary metabolites and small molecular compounds with broad insecticidal activity against most agricultural pests, with baveuricin being one of its most relevant toxins (WANG et al., 2021). Infection in insects depends on their physiological state, and the toxicity results from multiple compounds secreted by the fungus, with the same toxin varying in pathogenicity depending on the host (WANG et al., 2021).

As depicted in each bibliometric map, the discussion revolves around different topics of interest. In Figure 1, the red group highlights words related to the evolution and diversity of the Lepidoptera taxon. Studies suggest that lepidopteran studies may be more challenging than other insects due to their well-established monophyly by more basal groups, being structurally and ecologically very similar despite a diverse range of species (KRISTENSEN et al., 2007). Yellow keywords refer to pollination and the influence of nectar with compounds that deter or inhibit insect feeding, such as alkaloids and phenolic compounds (LANDOLT & LENCZEWSKI, 1993). The third group in blue is related to herbivory. The theme was discussed in studies exploring the potential of compounds or genes capable of inhibiting plant consumption, as in the case of passion fruit, while other works developed the relationship between the development and mortality of heliconian larvae with their host plants (BIANCHINI & MOREIRA, 2005; BOTELHO et al., 2008; BORGES et al., 2013). The fourth green cluster represents works that cover the phenology and migration of lepidopterans, exploring their seasonal patterns, migrant numbers, and related species (WALKER, 2001).

The organization of connected groups in Figure 2 highlights additional words for the species *D. juno*, and in red, works related to viroses, such as baculoviruses, which are among the most diverse groups. It is already known for its interaction with Lepidoptera families and the potential coevolution with its host population, being less virulent and specializing in specific hosts or, in extreme cases, causing speciation with its host (HERNIOU & JEHLE, 2008). Other works focused on discretionary characterization of genetic markers and taxonomic studies, with the *Heliconius lineage* being one of the most well-described and studied (MASSARDO et al., 2012). The yellow formation is related to *D. juno* interaction with passion fruit species, discussing the attractiveness of insects to different genotypes (ANGELINI & JUNIOR, 2007), as well as resistance and susceptibility of *Passiflora* species to *D. juno* (JUNIOR et al., 2008). On the other hand, the green formation of *D. juno* addresses studies that continue to explore the relationship of *D. juno* with passion fruit, such as tests of flavonoids for insect control (ECHEVERRI et al., 1991). The grouping contains studies exploring the biology of nymphalids and their diversity, with genetic description and taxonomy establishing *Dione* as an independent and sister lineage to *Agraulis*, discussing their structures and genetic markers (MASSARDO et al., 2015).

Regarding the development of research over time, it is noted that in the early 2000s, studies focused on the taxonomic and genetic description of insect species. Subsequently, around 2010, the topics shifted to focus on behavior and interactivity with passion fruit species, followed by biological control studies. In more recent works, speciation and diversities are discussed, while studies of the relationship between these insects and passionflower species continue, examining innate preferences and the influence of their morphology (DREWNIAK et al., 2020).

The association between fungi and passion fruit pests as a research area is still atypical. As demonstrated by bibliometric maps, the most regular research between *D. juno* and *A. vanillae* is taxonomic, ecological, and directly or indirectly involved with passion fruit cultivation. The growth of biocontrol studies brings the potential for further investigations into the interaction between fungi and insects (SILVA & MALTA, 2016). Given that *D. juno* and *A. vanillae* more regularly attack passion fruit crops, but at least twelve species of lepidopterans causing damage are registered, with eight belonging to Nymphalidae (LUNZ et al., 2006).

Currently, the most well-described relationship for the biological control of these insects is through their predation or control by parasitoids. This includes works reporting the collection and identification of natural enemies or laboratory tests, such as the model using *Palmistichus elaeisis*, implementing its possible in vitro breeding in *A. vanillae* (LIMA & VEIGA, 1995) (RODRÍGUEZ-DIMATÉ et al., 2016). One of the challenges for food demand is the need for technological innovations for crop protection while conserving natural resources. Furthermore, it is possible that the biological control technique is one that provides the most return on investment within Integrated Pest Management (NARANJO et al., 2015). Other benefits need to be monitored over time, such as classical control programs that have already been successful in reducing economic damage by reducing pest impacts and expenses with alternative control practices (NARANJO et al., 2015).

The *Fusarium* genus is widespread in nature, displaying saprobic habits or associations with plants and animals (TEETOR-BARSCH & ROBERTS, 1983). This group of fungi is known for possessing toxic metabolites and causing harm to its hosts, *Fusarium*, in insects, exhibits both pathogenic and non-pathogenic associations

(TEETOR-BARSCH & ROBERTS, 1983) (SANTOS et al., 2020). The pathogenicity of *Fusarium* has been confirmed for various orders of insects: Coleoptera, Thysanoptera, Hemiptera, Hymenoptera, Diptera, Lepidoptera; and its interaction is more diverse than concluded in previous works, but few studies explore its specificity with the host or its effects on non-target organisms (SANTOS et al., 2020). Regarding its association with Lepidoptera order organisms, *Fusarium* causes a low mortality rate in pathogenicity tests, despite being the second most studied order of insects (SANTOS et al., 2020). Another discussed relationship to justify the low infection levels in larvae is the accidental infection by *Fusarium* when larvae with already damaged cuticles come into contact with the fungus, subsequently infecting the leaves of plants consumed by these insects (HAJEK et al., 1993).

Insecticides are generally the most toxic group among pesticides, accumulating in natural food chains, being consumed in food or water (ALI et al., 2021). The effectiveness of biological control is not only in the mortality of insects but also takes into account the inhibition of herbivory on affected crops. In this sense, the replacement of chemical insecticides by microbial pathogens is still discussed (TEFERA & PRINGLE, 2003). It is believed that the virulence of fungal pathogens may be higher in insects without prior infection (MANTZOUKAS et al., 2022b). To achieve more sustainable systems and reduce the impact on non-target insects, additions and developments in biotechnology are necessary. Fungus-based pesticides can be modified to meet specific compatibility with target insects through the addition of genetic markers or silencers (NARANJO et al., 2015; MANTZOUKAS et al., 2022b).

The results suggest *Fusarium* sp. as a new fungal infectious agent for *A. vanillae*. The identification of *Fusarium* sp. as a potential entomopathogenic agent for *A. vanillae* in this study offers a novel contribution, though its confirmation via Koch's postulates is limited by methodological constraints. Four larvae were tested, with three exposed to the fungal isolate for 10 minutes on the culture medium and one maintained as a non-inoculated control, in which no pathogen was detected. While this supports the specificity of *Fusarium* sp. to the inoculated individuals, the small sample size and the brief, unquantified exposure method without standardized spore load or inoculum preparation restrict the robustness of these findings. The 10-minute exposure may not reflect natural infection dynamics, and the lack of spore quantification limits conclusions about the minimal infectious dose required for pathogenicity. These factors, combined with the use of a single control larva, only partially fulfill Koch's first postulate, which requires confirming the pathogen's absence across a broader population of healthy individuals. Given the opportunistic nature of this discovery and limited sample availability, these results are preliminary. Further studies with larger sample sizes, multiple controls, and controlled inoculation methods are essential to validate *Fusarium* sp. pathogenicity and assess its viability as a biocontrol agent in passion fruit crops.

5. CONCLUSIONS

Studies on fungal pathogens for the species *Dione juno* and *Agraulis vanillae* are still in their early stages, with well-known pathogens attacking other groups of insects or already used in the biological insecticide industry being recorded to date. *Fusarium* sp. is a new record for *Agraulis vanillae*, representing a fungus with potential for biological control. However, due to methodological limits, further research on this isolate and other fungal species is needed to develop biocontrol options for passion fruit pests, aiming to replace chemical insecticides.

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