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# Quantification and identification of damage caused by pests and fungi in dried figs from orchards with different levels of agronomic management in the main production areas of extremadura (SW Spain)



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#### ABSTRACT

This work aimed to evaluate the effect of agronomic management and water regime on the number of lesions, levels of insect infestation and microbiological quality of dried figs in Extremadura. Dried fig samples from 18 orchards were collected. The results showed that birds were the primary pests, causing damage to dried figs, followed by insects and fungi. The effects of orchard management were more pronounced under irrigated conditions, with the percentage of undamaged dried figs and the number of insect-free fruits rising significantly with increasing management. Under rainfed conditions, the level of orchard management did not significantly influence damage. In addition, insects were detected in both damaged and undamaged dry figs. *Cadra figulilella*, *Carpophilus hemipterus* and *Ceratitis capitata* were the most common species. Regarding mycobiota, orchard conditions did not significantly affect fungi counts, but they did influence species composition. *Aspergillus* spp. were predominant under all conditions, followed by *Alternaria* spp. under irrigated conditions. This work provides relevant information on the different biotic agents that affect dried figs, showing that a higher level of management under irrigated conditions reduces pest incidence. Such knowledge is essential for designing control methods to obtain higher quality fruits.

#### **1. Introduction**

The fig tree, *Ficus carica* L., which is closely associated with Mediterranean horticulture, was one of the first domesticated fruit trees in the world [\(Zohary and Spiegel-Roy, 1975](#page-10-0); [Weiss, 2015\)](#page-10-0). The world fig production was 1.34 million tons in 2021 [\(FAOSTAT, 2021\)](#page-9-0), being mainly concentrated in countries in the Mediterranean basin and the Middle East. Spain, which produced 60,190 t in 2021, is the sixth largest producer in the world and the leading producer in the European Union, with more than 60% of production localised in the Autonomous Community of Extremadura (SW Spain). In this area, almost 8000 ha are dedicated to this crop, producing more than 38,000 t annually [\(MAPA,](#page-9-0)  [2021\)](#page-9-0). Fresh figs are extremely perishable, and drying them has been the most widespread way to preserve them for a longer period ([Veberic](#page-10-0)  [et al., 2008\)](#page-10-0), thus facilitating their transport, storage and availability. Today, most commercially produced figs are dried or processed ([Flaishman et al., 2008](#page-9-0); [Shokoohi et al., 2022\)](#page-10-0).

Generally, crop management for dried fig production in Extremadura has been very traditional, involving minimal cultural practices. Under this system, irrigation, fertilisers or phytosanitary products are generally not applied, with only manure used at crop establishment. The trees are established in low-density orchards (100–150 trees/ha), maintaining a

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<span id="page-1-0"></span>large canopy size with very light pruning. and obtaining generally low yields (López-Corrales et al., 2011; [Flaishman et al., 2008\)](#page-9-0). The fruits are naturally dried by the sun; after ripening, the fig begins to partially dry on the tree, until it falls to the ground, where it completes its drying process ([Crisosto et al., 2011](#page-9-0)). Fruits are harvested from the soil two or three times during the summer season. In recent years, the search for more profitable production systems have led to more technological interventions, incorporating soil management, fertilisation, irrigation and pruning techniques in fig cultivation ([Jafari et al., 2022\)](#page-9-0), as well as the setting of high-density plantations (Galván [et al., 2021;](#page-9-0) Pereira et al., [2015\)](#page-10-0). The area of fig trees cultivated under irrigation in Extremadura has increased fivefold since 2015, reaching 18% of the total area in 2021 ([MAPA, 2021\)](#page-9-0). Consequently, different agronomic systems of crop management currently coexist in this production area.

The drying of the figs facilitates their preservation; however, this process does not guarantee a long shelf life because many biotic factors can spoil figs and cause important yield and quality losses [\(Flaishman](#page-9-0)  [et al., 2008](#page-9-0); [Villalobos et al., 2016;](#page-10-0) [Mat Desa et al., 2019\)](#page-9-0). Indeed, sun-drying figs in the open air can lead to losses of 30–40% of total production in developing countries ([Kumar et al., 2016\)](#page-9-0). Damage caused by birds can be significant in some regions. The starling (*Sturnus unicolor*) and the azure-winged magpie (*Cyanopica cyanus*) are the most common species of bird present in Spanish fig orchards [\(Casadomet](#page-9-0)  [et al., 2016\)](#page-9-0). Birds cause physical damage to fruit by sticking their beaks into ripe figs and then quickly open their mouths to eat the flesh ([Clark,](#page-9-0)  [1967\)](#page-9-0). After locating a fig crop, the birds become habituated to this food source, becoming a major problem ([Brien and Hardy, 2002](#page-9-0)). In addition to birds, several species of insects may feed directly on mature or dried figs, which is another factor causing alterations and loss of quality ([Simmons et al., 1931](#page-10-0); [Simmons and Nelson, 1975](#page-10-0); [Palmieri and Pereira,](#page-10-0)  [2018\)](#page-10-0). Insects colonise figs mainly through the ostiole, while the surface of the fruit remains intact [\(Shorey et al., 1989](#page-10-0); [Kong et al., 2013](#page-9-0)). Thus, fig varieties with smaller ostioles can be less susceptible to insects than those with larger ones ([Michailides, 2003\)](#page-9-0). Among insect pests of dried figs, one of the main pests is *Carpophilus hemipterus* (Linnaeus) (Coleoptera: Nitidulidae), which can penetrate fallen overripe fruits, with the subsequent development of larvae inside the fruit during the drying process [\(Simmons and Nelson, 1975](#page-10-0)). Other important insect pests are the moths *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) ([Erakay and Ozar,](#page-9-0)  [1979\)](#page-9-0), the former feeding inside the fruit, contaminating it with frass and silk produced by the larvae ([Damarh et al., 1998\)](#page-9-0).

These insect pests can also contribute to the distribution of fungal spores that cause fruit spoilage ([Buchanan et al., 1975;](#page-9-0) [Paster and](#page-10-0)  [Barkai-Golan, 2008;](#page-10-0) [Mostowfizadeh-Ghalamfarsa et al., 2022\)](#page-9-0). Specifically, dried figs are extremely susceptible to contamination by filamentous fungi. The major concern is toxigenic moulds, which under suitable environmental conditions can produce toxins that pose a health risk. The principal toxigenic fungi associated with dried figs are *Aspergillus* section *Flavi, Aspergillus* section *Nigri*, *Penicillium* spp. and *Alternaria* spp. [\(Javanmard, 2010](#page-9-0); [Heperkan et al., 2012;](#page-9-0) [Turkoz Bakirci,](#page-10-0)  [2020;](#page-10-0) Galván [et al., 2022a](#page-9-0), [2022b\)](#page-9-0), with aflatoxins and ochratoxin A being the most prevalent and harmful mycotoxins found [\(Bircan, 2009](#page-9-0); [Di Sanzo et al., 2018](#page-9-0); [Sulyok et al., 2020](#page-10-0); Galván et al., 2022a, [2023](#page-9-0)).

Traditional growing conditions and poor agricultural management in cultivation areas can favour increased contamination of sun-dried figs by insects and microorganisms ([Zorlugenç et al., 2008](#page-10-0)). The main biotic factors that degrade the quality of dried figs produced in traditional systems have been studied in other production areas [\(Thomas, 1979](#page-10-0); [Doster et al., 1996;](#page-9-0) [Burks and Brandl, 2005](#page-9-0)). However, there is limited knowledge about the role of these factors in fig orchards with intensive management (Berón [et al., 2020;](#page-9-0) Galván [et al., 2023](#page-9-0)). Therefore, this study aimed to evaluate the influence of the level of management of fig orchards in the production area of Extremadura under two water regimes (irrigated and rainfed) on the different types of damage to dried figs, as well as to assess the insect species and fungal microbiota. This

study is necessary to establish effective control strategies to reduce crop injury and guarantee the hygienic/sanitary quality of dried figs.

#### **2. Materials and methods**

# *2.1. Plant material and sampling*

Dried fig samples of the variety 'Calabacita' were collected during two consecutive seasons (2020 and 2021) from 18 commercial orchards located in the main production areas of Extremadura (SW Spain). The climate is Mediterranean, with very dry and hot summers, reaching maximum temperatures above 40 ◦C; winters can be mild and rainy or cold and dry [\(Moral et al., 2016](#page-9-0)). Total rainfall, temperature and relative humidity records during the experimental period are shown in Table S1 and Fig. S1 [\(REDAREX, 2023](#page-10-0)). Nine of the 18 orchards were in irrigated plantations, and the other nine were in rainfed plantations. In the irrigated plantations, 2500–3000  $m^3/h$ a of water were applied with self-compensating drippers of 4 L/h per tree in the centres of the rows, preventing wet areas where the figs fall, from May to September. Based on the degree of agronomic management of the orchards, they were classified into three levels of management: high, medium and low. The specific characteristics of each management level and orchard locations are indicated in [Table 1](#page-2-0). The dried fig sampling, inspections and determinations performed are graphically presented in Fig. S2. Specifically, in each orchard, 1 kg of dried figs was picked by hand directly from the ground between 15 and 31 August from five randomly selected sites per orchard, separated at least 30 m from each other. A total of 180 samples were collected (5 samples  $\times$  18 orchards  $\times$  2 seasons). The samples were transported under refrigeration to the laboratory for analysis.

#### *2.2. Assessment and quantification of damage to dried figs*

Dried figs were visually assessed one by one and classified as follows:

- a) undamaged fruits with no defects on the surface;
- b) bird pecks, fruit with a break in the longitudinal skin from the stalk to the ostiole and no pulp inside the fruit;
- c) visible insect damage, minor skin damage due to feeding or entry/ exit galleries caused by carpophages;
- d) moulds, dried figs that expelled powdery masses of spores through the ostiole from the interior or with clear evidence of developed mycelia;
- e) other types of damage, including damage caused by excess moisture and empty aborted fruit.

After sorting, the number of fruits in each category was counted, and the results were expressed as a percentage of the total number of fruits.

#### *2.3. Quantification of fruits infested by insects*

To determine the presence of insects inside the dried figs eligible for the industry, fruits classified as undamaged and visibly damaged by insects were examined. First, dried figs from the visible insect damaged category were isolated individually in jars and placed in a climatic chamber at 26  $\pm$  1  $^{\circ}$ C under a 16:8 h L/D photoperiod. These conditions were intended to be similar to those in the field (August), thus favouring larval development to reach adulthood. Jars were examined every 7 days. Emerging adults were extracted, labelled and frozen at − 80 ◦C. After three months, the remaining dried figs were dissected and observed under a stereomicroscope, checking for the presence of diapausing carpophagous insects. Regarding dried figs from the undamaged category, 50 fruits were randomly selected from each sample. These fruits were placed in transparent trays covered with a fine mesh that allowed ventilation and at the same time prevented insects from escaping. Next, the trays were incubated in a climatic chamber at 26  $\pm$ 

<span id="page-2-0"></span>Description of the agronomic management and specific location of the 18 fig orchards sampling in Extremadura (SW Spain).



<sup>a</sup> The nearest weather station to the location where humidity, temperature and rainfall data were recorded are shown in Fig.  $S1$  and Table S1.

<sup>b</sup> Winter fertilisation (Dec) with  $\sim$ 300 kg/ha NPK (9:18:27) and spring (May) fertilisation with ~100 kg/ha potassium nitrate. Soil nutrient analysis every 4–5 years.

 $\degree$  Only winter fertilisation (Dec) with  $\sim$ 300 kg/ha NPK (9:18:27).

1 ◦C under a 16:8 h L/D photoperiod. Fruits were examined daily, removing fruits with carpophagous larvae or signs of insect presence, such as faeces, webbing, injury or galleries. Afterwards, the undamaged figs that harboured insects inside were isolated individually and processed to favour their development as indicated above.

The results were expressed as the percentage of figs infested by insects. The total infestation estimate (TE) was calculated as the sum of the percentage of visible insect damaged category figs harbouring insects and the percentage of undamaged figs category harbouring insects using the following formula:

TE = (% fruits from the category: visible insect damage  $\times$  % infestation fruits from visible insect damage category)  $+$  (% fruits from the category: undamaged  $\times$  % infestation fruits from undamaged category)

#### *2.4. Identification of insects*

#### *2.4.1. Molecular identification using barcoding*

Genomic DNA from insects was extracted using the NucleoSpin DNA Insect mini kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA from each insect was amplified by PCR using the primers LCO1490 (5′-GGTCAACAAATCA-TAAAGATATTGG-3)' and HCO2198 (5′-TAAACTTCAGGGTGAC-CAAAAAATCA-3′) [\(Folmer et al., 1994](#page-9-0)). Amplifications were performed in a 50-μL reaction mixture containing 10 ng of DNA, 0.2 mM of each dNTP, 6.25 pmol of each primer, 0.1 vol of  $10 \times$  PCR buffer and 1.25 U DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). PCRs were run in a T100 thermal cycler (Bio-Rad Laboratories, Hercules, California, USA) with an initial denaturation at 95 ◦C for 3 min, followed by 35 cycles of 95 ◦C for 1 min, 58 ◦C for 1 min and 72 ◦C for 1 min, with a final extension period at 72 ◦C for 10 min. Amplification products were separated by electrophoresis in 1% agarose gels and visualised by staining with Midori Green Advance (Nippon Genetics, Tokyo, Japan). The PCR products obtained were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA) and sequenced by Macrogen, Inc. (Madrid, Spain). The sequences were edited using BioEdit 7.2.5 and checked by nucleotide BLAST comparison in the BOLDSYSTEMS database. The taxonomic identifications of the insects were determined based on the highest score.

# *2.5. Mould and yeast counts*

Microbial counts were performed on dried figs from the 18 orchards after sorting, using the categories established in section [2.2:](#page-1-0) undamaged, visible insect damage and bird pecks. Mouldy dried figs were not considered because of the high level of contamination. Under sterile conditions, a total of 15 g of flesh from several fruits was diluted in 135 mL of peptone water (Condalab, Madrid, Spain) and homogenised in a Stomacher 400 (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Ten-fold serial dilutions were made in peptone water, and 0.1 mL aliquots were inoculated onto potato dextrose agar plates (PDA agar, Condalab) acidified to pH 3.5 with a 10% (w/v) sterile tartaric acid solution. After incubating at 25 ◦C for 5 days, yeast and mould colonies were counted, and the results were expressed as log cfu/g.

## *2.6. Isolation and identification of moulds*

#### *2.6.1. Mould isolation*

Three mould colonies were randomly taken from the highest dilutions of each acidified PDA plate after counting. The isolates were subcultured onto acidified PDA plates until a pure culture was obtained. In the case of dried figs classified as mould-damaged, each fruit was individually diluted in 40 mL of peptone water, and 0.1 mL was inoculated on acidified PDA agar plates. After incubation at 25 ◦C for 5 days, a mould colony with the predominant morphology was isolated from each PDA plate as above. Spores of each pure mould isolate were obtained by scraping the mycelium with 10 mL of sterile distilled water containing 0.05% (v/v) Tween 80 (Scharlab, Barcelona, Spain). The spore suspension of each pure mould isolate was stored at −80 °C in glycerol solution (50% v/v) until use.

#### *2.6.2. Mould identification*

To extract the genomic DNA, mould isolates were grown on PDA agar

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at 25  $\degree$ C for 7 days, and then a portion of approximately 0.5 cm<sup>2</sup> of the mycelium was removed aseptically with a scalpel and deposited in a tube with beads. Genomic DNA from each isolate was extracted using the quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, California, USA) according to the manufacturer's instructions. DNA concentration and purity were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

To identify mould isolates at the species level, the internal transcribed spacer ITS1/ITS2-5.8 S rDNA and partial β-tubulin gene were amplified using the primer pairs ITS1/ITS4 (ITS1: 5′-CTTGGTCATTTA-GAGGAAGTAA-3; ITS4: 5′-TCCTCCGCTTATTGATATGC-3'; [White et al.,](#page-10-0)  [1990\)](#page-10-0) and Bt2a/Bt2b (Bt2a: 5′-GGTAACCAAATCGGTGCTGCTTTC-3'; Bt2b: 5-ACCCTCAGTGTAGTGACCCTTGGC-3'; [Glass and Donaldson,](#page-9-0)  [1995\)](#page-9-0), respectively. Each PCR reaction was performed in a 50-μL reaction mixture containing the reagents and concentrations indicated above. PCR was run in a T100 thermal cycler (Bio-Rad Laboratories) with an initial denaturation at 94 ℃ for 4 min, followed by 35 cycles of 94 ℃ for 1 min, 55 ℃ for 1 min and 72 ℃ for 1 min, with a final extension period at 72 ◦C for 10 min. Amplification products were visualised, purified and sequenced as above. The sequences were edited with BioEdit 7.2.5 and checked by nucleotide-nucleotide BLAST comparison with the NCBI database. The taxonomic identifications of the isolates were determined based on the highest score.

#### *2.7. Statistical analysis*

The influence of agronomic management (irrigation factor with two levels: Irrigated and Rainfed; and crop management factor with three levels: High, Medium and Low) on the type of fruit damage, insect damage and microbiological data were evaluated by analysis of variance (ANOVA) using SPSS for Windows, 25.0. The season was considered as an additional independent and categorical factor in the ANOVA (threeway ANOVA). To evaluate the influence of damage category (undamaged, visible insect damage and bird pecks) on the mould and yeast counts obtained individually for each factor (water regime and management level), a one-way ANOVA was developed. Subsequently, the Bonferroni post hoc test was applied to compare the mean values obtained, and the significance level was set at  $p \leq 0.05.$  The percentage of mould isolates identified at the genus level was evaluated using Monte Carlo chi-square tests using contingency tables. Statistically significant differences were established by Bonferroni post hoc test. Finally, principal component analysis (PCA) was performed to evaluate the impact of agronomic management on the species of mould that occurred.

# **3. Results and discussion**

#### *3.1. Impact of agricultural management on dried fig damage*

The ANOVA results are shown in Table 2. The mean percentage of undamaged dried figs under rainfed conditions ranged from 79.22 to 81.24%, with no significant difference ( $p > 0.05$ ) between the crop management levels ([Table 3](#page-4-0)). In contrast, the percentage of undamaged dried figs was significantly affected ( $p \leq 0.05$ ) by the crop management level under irrigated conditions, with values ranging from 57.65 to 91.34% for low and high crop management, respectively. Figs that fall on moist surfaces may absorb external moisture, which delays their drying time  $(Sen, 2022)$  $(Sen, 2022)$  and prolongs the environmental exposure of wilted fruit. These conditions may favour an increase in fruit damage caused by pests ([Flaishman et al., 2008](#page-9-0)). Accordingly, these data suggest that being a low-management orchard with an irrigation system is an aggravating factor that increases the percentage of damaged fruit by birds and insects.

The main cause of dried fig damage was bird pecks. The mean percentage of this type of damage under rainfed conditions ranged from 8.55 to 11.72%, with no significant difference  $(p > 0.05)$  between crop management levels. However, the mean percentage of damage in



Error 168

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<sup>a,b,c</sup> Indicate significant differences  $p \le 0.05$  between the crop management levels with the same water management regime.<br><sup>1,2</sup> Indicate significant differences  $p \le 0.05$  between water management regimes with the sam

irrigated orchards ranged from 1.13 to 33.39% for high and low levels of management, respectively, decreasing significantly ( $p \leq 0.05$ ) as the level of crop management increased. In addition, at the same level of crop management (low or high), significant differences ( $p \leq 0.05$ ) were recorded between rainfed and irrigated orchards. The data show that the percentage of damage caused by birds increased strongly under irrigated conditions as the level of crop management decreased, whereas, under rainfed conditions, it remained unchanged. Several studies also point to bird pecks as the main cause of damage to figs in irrigated orchards. [Pereira et al. \(2015\)](#page-10-0) found bird damage between 11 and 30% of fresh fig and breba crops (fruit set on wood from the previous year), while [Galv](#page-9-0)án [et al. \(2021\)](#page-9-0) attributed between 29.3 and 55.7% of all damage recorded in dried figs to birds.

Fig orchards are generally located close to areas where water is available, hence there are areas of native vegetation that provide habitat for birds. In warm periods, irrigation can also influence the number of birds present in the crops; starlings have been observed to move around depending on the vineyard blocks being irrigated [\(Tracey et al., 2007](#page-10-0)). A good bird-repellent system, whether involving netting, sound or visual deterrents, can be important to increase production. The effectiveness of bird repellent techniques can vary by regions, species and fruit crops ([Simon, 2008](#page-10-0)). The most widespread and effective method in fig crop is the use of a physical barrier such as nets [\(Singh et al., 2022](#page-10-0)). However, in Extremadura, this method is not generally used due to the large canopy of the trees and its high cost. In this area, different sound scaring and visual bird deterrents strategies are commonly employed, showing the best results when both are combined [\(Bishop et al., 2003;](#page-9-0) [Tracey](#page-10-0)  [et al., 2007;](#page-10-0) [Simon, 2008\)](#page-10-0). The next most important surface damage recorded was caused by insects. Similar mean values, around 5%, were found for irrigated and rainfed orchards. These percentages are similar to those reported by [Burks and Brandl \(2005\),](#page-9-0) who found that 8.6% of samples of dried figs from California were infested with insects. However, as for bird damage, the extent of damage was influenced by water and crop management. In particular, no significant differences (*p >* 0.05) were found between the different crop management levels in rainfed orchards, while in irrigated orchards, higher crop management resulted in a significant decrease in damage ( $p \leq 0.05$ ). The lowest average percentage of visible insect damage (1.81%) was found on dried figs under a high level of crop management in irrigated orchards. A higher crop management implies a higher number of harvests during the season ([Table 1\)](#page-2-0), which involves a shorter exposure time of figs to biotic and abiotic factors that can impact fig production. This fact may explain that in our study a high crop management of dried figs had lower levels of damage. Therefore, these data suggest that minimising the time between harvests may be a suitable strategy to limit insect infestation and proliferation. Regarding dried fig damage by clear mould proliferation, the average percentages obtained were lower than 1.3% in all conditions studied, with an average of 0.87% damage in rainfed orchards and 0.70% damage in irrigated orchards. As with the dried fig damage reported above, there were no significant differences ( $p > 0.05$ ) between the different crop management levels in rainfed orchards, while in irrigated orchards, surprisingly, less crop management led to a lower incidence of mouldy dried figs ( $p \leq 0.05$ ). A low incidence of damage

caused by mould was reported by  $Galván et al. (2021)$  $Galván et al. (2021)$ , who found in the Extremadura production area mould damage in 1.64–5.50% of fruits in a high-density (1000 trees/ha) irrigated plantation, depending on year. Finally, other damage (not classified as mould/insect/bird damage) presented average percentages of 4.09% in rainfed orchards and 3.77% in irrigated orchards. These types of damage were not affected by the level of crop management in irrigated orchards; however, in rainfed orchards, low management resulted in the lowest mean damage (*p* ≤ 0.05). Overall, the level of crop management had a minimal impact on the amount of dried fig damage in rainfed orchards, whereas in irrigated orchards a high level of crop management reduced the incidence of damage. Concerning the effect of the season factor, its influence on damage classification under the conditions studied was generally low (Table S2). The condition in which a greater influence of season was observed was rainfed with low management, where 3 of the 6 damage categories (undamaged, bird pecks and mould) showed significant differences ( $p \leq 0.05$ ).

### *3.2. Dried fig infestation by insect pests*

The average rate for undamaged and insect-infested dried figs was 4.26%, with no significant differences (*p >* 0.05) between the water and crop management regimes ([Table 4](#page-5-0)). During sorting, fruits with significant damage, such as bird pecks, are removed, but fruits with insect damage, being minor and not easily detectable, remain during the industrial processing of dried figs and may appear and become conspicuous later during commercialisation. These insects can continue to degrade the quality of the dried figs during storage by the farmer until they are fumigated with phosphine before being processed industrially. Heavy infestations can cause serious alterations in dried figs that make the product commercially unacceptable ([Eliopoulos and Athanassiou,](#page-9-0)  [2003;](#page-9-0) [UNECE, 2016](#page-10-0)). As expected, for fruits classified as "visible insect damaged", the percentage of insect-infested fruit was noticeably higher than in fruit from the undamaged category. The average values obtained were highly variable depending on the water and crop management conditions in the orchards, although no significant differences ( $p > 0.05$ ) were observed between irrigated and rainfed orchards with medium and high crop management levels. However, with low management, the percentage of fruit with surface damage that was infested by insects was significantly higher in irrigated orchards, showing the highest mean value of 55.66%, whereas, in rainfed orchards, the percentage was only 7.46%. Dry environments may favour the proliferation of some generalist species ([Arenas-Clavijo and Armbrecht, 2018\)](#page-9-0), such as ants ([Burks](#page-9-0)  [and Brandl, 2005\)](#page-9-0) or the coleopteran *Gonocephalum pusillum* Fabricius (Coleoptera: Tenebrionidae) ([Gragera-Facundo, 2014](#page-9-0)), that cause superficial damage to dry figs without infesting them.

Finally, the estimated mean percentage of dried figs infested by insects out of the total figs harvested was higher in irrigated orchards (4.80%) than in rainfed plantations (3.70%), and the difference was significant ( $p \leq 0.05$ ) at the low level of crop management. Crop management had a significant influence ( $p \leq 0.05$ ), although its influence differed between rainfed and irrigated orchards. In the case of irrigated orchards, the mean percentage of infested fruit increased as the level of

<span id="page-5-0"></span>



<sup>a,b,c</sup> Indicate significant differences  $p \le 0.05$  between the crop management levels with the same water management regime.<br><sup>1,2</sup> Indicate significant differences  $p \le 0.05$  between water management regimes with the sam

crop management decreased, showing significant differences between high and low levels of management ( $p \leq 0.05$ ), with values of 3.08 and 6.70%, respectively. Differences between seasons were only significant  $(p \le 0.05)$  in both water regimes at a medium management level (Table S2). These findings show that in irrigated orchards, high levels of crop management help to reduce the presence of insects inside dried figs. In contrast, in rainfed orchards, differences were not significant between crop management levels ( $p > 0.05$ ).

Dried fig production takes place in summer, when it is extremely dry and hot in Extremadura ([Moral et al., 2016\)](#page-9-0). The average daily relative humidity (RH) and temperature were 42.9% and 26.1 ◦C during July and August of both years studied (Fig. S1). However, irrigation in crops affects the microclimate of the environment by increasing humidity and reducing the temperature ([Rosenberg, 1974;](#page-10-0) [Sun et al., 2022\)](#page-10-0). These conditions may favour the development of known pests of dried figs, such as *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *C. hemipterus* and *Cadra figulilella* (Gregson) (Lepidoptera: Pyralidae), as they are close to their optimum relative humidity of 60–70% [\(Shoukry](#page-10-0)  [and Hafez, 1979](#page-10-0); [James and Vogele, 2000](#page-9-0); [Cox, 1974](#page-9-0)). In addition, irrigation promotes the emergence of weeds ([Verma et al., 2015](#page-10-0)), which can become a reservoir of insect pests and diseases in orchards where weeds are not controlled (low management) ([Kumar et al., 2021\)](#page-9-0). The frequency of harvesting under irrigation conditions may therefore influence the level of dried fig infestation by insects. By contrast, in rainfed production, dried figs are exposed to higher temperatures. According to [Simmons et al. \(1931\)](#page-10-0), figs can reach maximum temperatures of 55 °C during sun drying, which has lethal effects on different life stages of *C. hemipterus* (larvae and eggs). [Shorey et al. \(1989\)](#page-10-0) assessed the influence of solar radiation on insect infestations in dried figs, finding that higher temperature and exposure to direct sunlight reduces insect infestations from 4.2 to 0%, preventing new infestations in dried figs on the ground for at least 10 days. This evidence may explain why, in rainfed orchards in the Extremadura area, the level of management has a lower influence on insect infestation than in irrigated orchards.

# *3.3. Identification and quantification of insect species*

Nine insect taxa were identified as pests inside the dried figs collected, belonging to the orders Lepidoptera, Coleoptera, Diptera and Hymenoptera (Table 5). A smaller number of insects that could not be identified by DNA barcoding were classed as "other species".

Two common Lepidoptera species, *C. figulilella* and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), previously known as dried fig pests [\(Ferguson et al., 1990; Ben-Yakir and Costa, 2022\)](#page-9-0), were found in infested fruits from all crop management systems (rainfed and irrigated) at different rates. *C. figulilella* was the most common insect identified, being found in 44.54 and 26.1% of infested figs in rainfed and irrigated orchards, respectively. However, the exact percentage varied depending on the water and crop management regime, being highest in rainfed and irrigated orchards with low and high levels of management, respectively. These results are consistent with those described by [Donohoe et al. \(1934\)](#page-9-0) and [Jalili et al. \(2004\)](#page-9-0) in different pest management studies on dried figs, in which *C. figulilella* was found to be the main cause of damage and losses after harvest, especially during drying and early storage. By contrast, [Burks and Brandl \(2005\)](#page-9-0) found that the predominant lepidopteran on dried figs was *Amyelois transitella* Walker (Lepidoptera: Pyralidae), which was responsible for 32% of the total infestation. The incidence of the other Lepidoptera identified in all orchard conditions studied was more homogeneous, with values ranging from 5.6 to 11.1%.

Another commonly found lepidopteran was *Cadra abstersella* (Zeller) (Lepidoptera: Pyralidae) which was identified in most of the management regimes, except rainfed orchards with low levels of management. Its incidence was similar in different types of irrigated orchards (4.6–14.7%); however, it was the dominant species at 47.2% in rainfed orchards with medium management. As far as we know, this moth has never been described as a fig pest. Other Lepidoptera identified were *Cryptoblabes gnidiella* (Milli`ere) (Lepidoptera: Pyralidae) and *Ephestia parasitella* Staudinger (Lepidoptera: Pyralidae). Both species were found in irrigated orchards with low management, and *E. parasitella* was also found in the rainfed system with high management. Neither moth has been previously recorded as a pest of dried figs, but both are known pests of dry fruits, such as raisins [\(Xuereb et al., 2003](#page-10-0); [Elnagar, 2018](#page-9-0)), and *C. gnidiella* has been even found damaging acorns [\(Torres-Vila et al.,](#page-10-0)  [2002\)](#page-10-0).

One of the most important coleopteran pest species of figs is *C. hemipterus*, which was associated with dried figs in California in the early 20th century by [Simmons et al. \(1931\)](#page-10-0). This Nitidulidae beetle was







detected in most of the production systems studied, except in rainfed systems with medium management. The percentage detected was substantially higher in irrigated than in rainfed orchards, at 32.40% versus 1.26%, respectively. These percentages are lower than those reported by [Burks and Brandl \(2005\)](#page-9-0) in California plantations, where 53% of the infestation in dried figs was attributed to Nitidulidae species.

Another insect pest of figs identified in the current study was *C. capitata*. The Mediterranean fruit fly was detected in rainfed and irrigated orchards under low and high levels of management. Its incidence was variable, being highest in rainfed orchards with low management, where it was responsible for 46.7% of infested fruit. In a fruit fly management study by [Howell et al. \(1975\),](#page-9-0) an average of 4.77% of the total fresh fig harvest was infested with *C. capitata* larvae in the absence of treatment.

Another insect species with low occurrence was *Drosophila simulans*  Sturtevant (Diptera: Drosophilidae), found in 7% and 1.1% of infested figs in irrigated orchards with high and low levels of management, respectively. The problem associated with these flies is not so much the direct damage they cause as the fermentation they induce, altering the quality of the fruit with soft and wet spots ([Casadomet et al., 2016\)](#page-9-0).

Finally, one of the most interesting insects identified in this study was the hymenopteran *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). The percentage of fruits harbouring this species was low, ranging from 0.41 to 2.56% depending on the level of crop management. This parasitoid wasp successfully attacks the larvae of several lepidopteran species belonging to the family *Pyralidae*. The insect can be used to control fig moths during the storage of dried figs as a biocontrol agent in an integrated pest management programme [\(Suma et al., 2014](#page-10-0)). In general, a higher insect species diversity was observed in fruits from irrigated orchards than from rainfed orchards, and changes in temperature and humidity are known to alter insect diversity ([Majeed et al.,](#page-9-0)  [2022\)](#page-9-0). This knowledge provides preliminary information for future studies to improve integrated pest management (IPM) programmes in dried fig processing.

#### *3.4. Mould and yeast quantification*

Mould and yeast counts for damaged and undamaged dried figs from the different water and crop management regimes are shown in Fig. 1. The mean counts ranged from 2.22 to 3.36 log cfu/g for moulds and from 2 to 3.09 log cfu/g for yeasts. These data are consistent with those reported by [Villalobos et al. \(2019\)](#page-10-0) and Galván [et al. \(2023, 2022a\)](#page-9-0) for sun-dried figs of the same cultivar before industrial processing. Overall, these results show that the variables studied (water and crop management) did not significantly influence mould and yeast counts among figs in the same damage category  $(p > 0.05)$ , except in the case of mould counts for figs with insect damage and medium management, where significant differences were found between irrigated and rainfed conditions ( $p < 0.05$ ). However, the mean mould counts were lower in undamaged and visible insect-damaged dried figs from orchards with high levels of management, both irrigated and rainfed, than in the rest of the regimes ( $p > 0.05$ ). This tendency could be due to the importance of orchard sanitation (such as pruning, land preparation and Bordeaux mixture treatments in winter) and moisture control through canopy management and irrigation as methods of cultural control of fruit diseases [\(Mostowfizadeh-Ghalamfarsa et al., 2022](#page-9-0)). This is important, since mould contamination and mycotoxin occurrence are some of the main problems associated with the production of dried figs (Galván et al., [2022a\)](#page-9-0), and suitable management may help to limit this. In addition, fig skin is soft and can be easily physically damaged, as well as destroyed by fungal growth and subsequent mycotoxin production ([Heperkan et al.,](#page-9-0)  [2012\)](#page-9-0). However, in this study, there was no significant difference (*p >* 0.05) between dried figs classified as undamaged and visible insect-damaged with the same water management regime and management level, with mean mould counts of 2.47 and 2.35 log  $\text{cfu/g}$ , respectively. In contrast, figs damaged by bird pecks had the highest





**Fig. 1.** Mean counts  $\pm$  SD (log cfu/g) of moulds (A) and yeasts (B) in dried figs classified as undamaged (green), insect-damaged (orange) and bird-damaged (blue) from different levels of crop management and water regime. <sup>a,b,c</sup> Indicate significant differences  $p \leq 0.05$  between undamaged fruit and different types of fruit damage with the same water management regime and management level. \*Indicates significant differences  $p \leq 0.05$  between water management regimes within the same management level.

mean mould counts in all conditions studied. However, the differences were only significant ( $p \leq 0.05$ ) in rainfed for high and low management and in irrigation for low management. Birds can be vectors of fungal diseases found on the fruit ([Tracey et al., 2007\)](#page-10-0), and after being pecked, the entire interior of the fruit is exposed to the environment, facilitating contamination by microorganisms.

# *3.5. Identification of moulds*

Dried figs are susceptible to being contaminated by different species of moulds, which may compromise their quality and mycotoxin contamination ([Sulyok et al., 2020](#page-10-0); Galván [et al., 2022a](#page-9-0)). A total of 836 moulds were isolated and identified, 452 from rainfed orchards and 384 from irrigated orchards. A total of 47 species were identified (Table S3), 89.47% of which belonged to the genera *Aspergillus*, *Alternaria*, *Penicillium*, *Cladosporium*, *Talaromyces* and *Fusarium*. [Table 6](#page-7-0) shows the relative abundance of mould genera based on crop management level (low, medium and high) and fruit damage classification (undamaged and damaged by birds, mould or insects) in rainfed and irrigated orchards. The genus *Aspergillus* was the most prevalent under both water management regimes, with significant differences ( $p \leq 0.005$ ) between rainfed (73.5%) and 56.3% in irrigated systems. Most species of *Aspergillus* are resistant to water stress, being adapted to hot and dry conditions, and continue to grow during fruit drying ([Flaishman et al., 2022](#page-9-0)). In this regard, water stress together with high temperatures are more suitable conditions for colonisation by aflatoxin-producing *Aspergillus*  spp. [\(Bircan et al., 2008; Marroquín-Cardona et al., 2014](#page-9-0)). A total of 16 species of this genus were identified, with a high prevalence of

<span id="page-7-0"></span>Relative abundance (%) of mould genera on damaged and undamaged dried figs from different management levels.



<sup>a,b,c</sup> Indicate significant differences  $p \le 0.05$  between the crop management levels with the same water management regime.<br><sup>1,2,3</sup> Indicate significant differences  $p \le 0.05$  between undamaged fruit and different types

\*Indicates significant differences *p* ≤ 0.05 between water management regimes.

*A. tubigensis, A. welwitschiae, A. niger* (section *Nigri)* and *A. flavus* (section *Flavi*). A high incidence of *Aspergillus* spp. has been reported in different studies of dried figs throughout all stages of the production process ([Javanmard, 2010](#page-9-0); Galván [et al., 2022a](#page-9-0), [2023\)](#page-9-0).

In contrast, the incidence of *Alternaria* spp. was significantly higher  $(p < 0.05)$  in figs produced in irrigated orchards than in rainfed orchards, at 16.6% and 3.5% respectively. Galván [et al. \(2023\)](#page-9-0) studied two orchards with different water management regimes and observed a lower proportion of *Alternaria* spp. in rainfed figs (7.1%) than in irrigated figs (12%). The higher ambient humidity in orchards due to irrigation may favour the spread of *Alternaria* spp. [\(Lee et al., 2015](#page-9-0)). Likewise, the genus *Talaromyces*, which is not commonly associated with dried fig mycobiota, was more abundant in irrigated areas. Finally, the incidence of *Penicillium* and *Cladosporium*, which are considered highly ubiquitous and easily spread fungal species ([Egbuta et al., 2016](#page-9-0)), did not differ significantly between water regimes (*p >* 0.05).

Regarding the crop management level, the data show that in rainfed orchards, it had no significant impact (*p >* 0.05) on the three most prevalent genera, *Aspergillus*, *Penicillium* and *Alternaria*. On the contrary, in irrigated orchards, significant differences ( $p \leq 0.05$ ) were found between crop management levels. In particular, higher crop management led to a significant increase in the relative abundance of *Aspergillus* spp. This is noteworthy because this genus encompasses the main mycotoxinproducing species associated with dried figs ([Taniwaki et al., 2018](#page-10-0)). However, lower crop management led to a significant increase in *Penicillium* spp., *Alternaria* spp. and *Cladosporium* spp. This impact of management on mycobiota in irrigated orchards may be due to the irrigation strategy. A high level of management leads to greater control of irrigation by avoiding water accumulation on the soil surface and high moisture levels that would interfere with fruit drying. Inadequate irrigation management during the harvest period can lead to an increase in fungal diseases that find the optimum environmental conditions for their proliferation [\(Aksoy, 1981](#page-9-0); [Can, 2022](#page-9-0)).

Concerning the mycobiota of undamaged and physically damaged (from birds and insects) dried figs, no significant differences (*p >* 0.05) were observed for the genera *Aspergillus* and *Penicillium* in dried figs from rainfed orchards; however, dried figs under irrigation showed significant differences ( $p \leq 0.05$ ) in the incidence of these genera. Physically damaged dried figs from irrigated orchards were more susceptible to mould contamination by *Aspergillus* and *Penicillium*. The species of these genera form large numbers of conidia on conidiophores

and can be easily distributed by birds, which are more common in irrigated orchards ([Mcginness et al., 2015](#page-9-0)). The opposite dynamic was observed for *Alternaria* spp., which was detected at its highest level in undamaged dried figs from both water management systems, in 14.3 and 44.4% of figs from rainfed and irrigated orchards, respectively. It has been reported that wounds on very ripe figs do not increase infection and subsequent aflatoxin contamination, and that insect damage does not predispose the fruit to infection ([Michailides, 2003](#page-9-0)). However, birds and insects can be vectors of entry by transmitting the spores of these fungi. [Phillips et al. \(1925\)](#page-10-0) showed that the pests *C. hemipterus* and *Drosophila melanogaster* can transmit fungal spores to edible figs, and [Michailides et al. \(1991\)](#page-9-0) reported that beetles (*Carpophilus* spp.) transmitted *Aspergillus niger* to healthy 'Calimyrna' figs when the spores were mixed with soil dust.

In the case of dried figs damaged by mould, *Aspergillus* spp. clearly predominated ( $p \leq 0.05$ ) in both water management regimes, being found in 93.8 and 86.0% of mouldy figs from rainfed and irrigated orchards, respectively. The adaptation of the species of this genus to the physicochemical characteristics and nutritional composition of dried figs has been previously reported [\(Taniwaki et al., 2018](#page-10-0)). These findings are consistent with those recorded in California by [Doster et al. \(1996\)](#page-9-0), who attributed mould rot to *Aspergillus niger* and *Aspergillus welwitschiae*  species in 93% of the 'Calimyrna' figs and 99% of the 'Conadria' figs. These mould species, which usually contaminate dried figs with ochratoxin A, must be removed and disposed of in separate containers to avoid cross-contamination by the dissemination of spores in collection and transport containers (Sen, 2022). In most industrial processing units, along with mouldy and defective figs, figs that emit bright greenish-yellow fluorescence are also removed under UV light. This technique is commonly used to detect figs contaminated with aflatoxins ([Heperkan et al., 2012](#page-9-0); [Mat Desa et al., 2019](#page-9-0)).

The PCA loadings and scoring plots summarise the identified mould species according to the crop management level and type of damage ([Fig. 2](#page-8-0)). The results of the factor analysis showed a clustering of the samples along PC1 and PC2, explaining 21.29 and 13.59% of the variance, respectively. Samples from rainfed orchards were located on the left side of PC1 ([Fig. 2A](#page-8-0)); this behaviour is explained by the negative loading on the same axis ([Fig. 2B](#page-8-0)) of most species of the genus *Aspergillus*. On the contrary, samples from irrigated orchards were located on the right side of the PC1 axis ([Fig. 2A](#page-8-0)), which is attributed to the higher presence of *Alternaria* spp. It can also be seen that samples from rainfed

<span id="page-8-0"></span>

PC2 13.59%  $\bigotimes_{\text{Al}} 0.8$  $-0.8$  $-0.6$  AFI  $-0.4$ TAn  $0.2$  $0.4$  $PM^{\overline{0}...}$  $06$  $-$  PH  $_{2}$ CHa  $x$  PRi  $TPi$ – ÄEu Alb<sup> $\triangle$ </sup>ACh<br> $\frac{1}{2}$ C<sub>n</sub>  $-0.4$ PMe  $\bullet$  CO<sub>2</sub> **X**PCr<br>XATe  $\bullet$  FOx  $-0.6$ PS  $-0.8$ PC1 21.29 % **Fig. 2.** Projection of samples grouped according to water regime, crop management and classification of fruits (A) and mould species identified (B) in the

space defined by the first two components (PC1/PC2): *Alternaria eureka* (AE), *Alternaria section Alternaria* (AA), *Alternaria section Infectoriae* (AI), *Alternaria*  section *Ulocladioides* (AU), *Aspergillus alliaceus* (Aal), *Aspergillus amstelodami*  (AAm), *Aspergillus chevalieri* (ACh), *Aspergillus europaeus* (AEu), *Aspergillus flavus*  (AFl), *Aspergillus fumigatus* (AFu), *Aspergillus ibericus* (AIb), *Aspergillus melleus*  (AMe), *Aspergillus niger* (ANi), *Aspergillus parasiticus* (APa), *Aspergillus tamarii*  (ATa), *Aspergillus terreus* (ATe), *Aspergillus tubingensis* (ATu), *Aspergillus udagawae* (AUd), *Aspergillus uvarum* (AUv), *Aspergillus welwitschiae* (AWe), *Cladosporium halotolerans* (CHa), *Cladosporium oxysporum* (COx), *Fusarium oxysporum*  (FOx), *Penicillium bilaiae* (PBi), *Penicillium brevicompactum* (PBr), *Penicillium citrinum* (PCi), *Penicillium crustosum* (PCr), *Penicillium expansum* (PEx), *Penicillium hispanicum* (PHi), *Penicillium melanoconidium* (PMe), *Penicillium menonorum*  (PMe), *Penicillium sizovae* (PSi), *Talaromyces amestolkiae* (TAm), *Talaromyces pinophilus* (TPi), *Talaromyces purpureogenus* (TPu), *Talaromyces trachyspermus*  (TTr), other (O).

orchards, except for those with mould damage, are concentrated in the same part of the axis of PC1 and PC2, while those from irrigated orchards are distributed in both parts of the axes. These results provide further support for the hypothesis that the mycobiota are affected by the conditions generated by water management. The results of the factorial analysis according to the level of crop management (high, medium and low) showed an influence on the mycobiota in the case of irrigated orchards, with high management on the left of the PC1 axis (Fig. 2A) and low management on the right of the axis. Finally, in the case of damaged figs, the samples with mould damage from both water management regimes are located on the left side of the PC1 axis (Fig. 2A), while *Aspergillus welwitschiae* and *Aspergillus niger* are located on the negative side of the PC1 axis (Fig. 2B).

#### **4. Conclusions**

Our results show that birds were the main cause of dried fig damage in all areas of Extremadura, followed by insects and moulds. A high level of orchard management under irrigated conditions led to a considerable reduction in the percentage of damaged fruit. It is worth noting that pest insects were detected in damaged and undamaged dried figs, with their prevalence being significantly influenced by the level of management in irrigated orchards. Among the nine insect species identified, *C. figulilella*, *C. hemipterus* and *C. capitata* were the most widespread. Regarding mycobiota, fig orchard conditions did not significantly affect counts, but influenced species composition. *Aspergillus* spp. were dominant under rainfed conditions, while *Alternaria* spp. predominated in irrigated orchards. Therefore, our work shows the importance of agronomic management on insects, birds, and fungi, suggesting that a higher management level under irrigation conditions reduces pest incidence. This fact may be even more critical today, as we move from more traditional rainfed to more intensive irrigated orchards. At the same time, it provides relevant information on the different biotic agents that may interfere with dried figs in different cropping scenarios. This knowledge is essential when designing phytosanitary control/management methods to obtain fruits of higher hygienic-sanitary quality. However, further studies considering the different agronomic factors independently are needed to better quantify the individual impact of each factor.

# **CRediT author statement**

A.J. Galán: Conceptualisation, Investigation, Formal analysis, Writing–original draft. A. Martín: Methodology, Formal analysis, Writing–review & editing. L.M Torres-Vila: Methodology, Visualisation, Supervision, Writing–review & editing. S. Ruiz-Moyano: Methodology, Supervision, Writing–review & editing. A.I. Galván: Methodology, Formal analysis, Investigation. M.J. Serradilla: Methodology, Visualisation. M. Lopez-Corrales: Conceptualisation, Writing–review & editing, Project administration, Funding acquisition.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# **Data availability**

Data will be made available on request.

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#### **Appendix A. Supplementary data**

Supplementary data to this article can be found online at [https://doi.](https://doi.org/10.1016/j.cropro.2023.106334)  [org/10.1016/j.cropro.2023.106334](https://doi.org/10.1016/j.cropro.2023.106334).

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