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Identificación y control de agentes bióticos causantes de alteraciones en el proceso productivo del higo seco

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Identification and control of biotic agents causing alterations in the production process of dried figs

ANTONIO JESÚS GALÁN JIMÉNEZ

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RESUMEN

RESUMEN

El objetivo de este estudio fue analizar los agentes bióticos que afectan al higo seco en Extremadura, así como evaluar la eficacia de sistemas de control durante el proceso productivo que permitan minimizar los daños causados por las mismas. Para ello, se tomaron muestras de higos secos de 18 higuerales con diferentes niveles de manejo siendo las aves la principal causa de daños, seguidos de insectos (*Cadra figulilella*, *Carpophilus hemipterus* y *Ceratitis capitata*) y mohos (*Aspergillus*). Además, en los higuerales de regadío las labores agrícolas adquieren mayor relevancia para el control de plagas. En este contexto, en el campo durante la maduración y secado del fruto, el uso del sistema de mallas adaptado a la copa redujo significativamente los daños causados por aves e insectos del orden Lepidóptera, así como la contaminación por micotoxinas, mejorando además características fisicoquímicas: peso del fruto, color y firmeza. En la industria, los tratamientos de congelación (-18ºC 1 día, -10ºC 2 días o -5ºC 7 días) mostraron ser una alternativa eficaz a la fosfina en el control de insectos manteniendo la calidad sensorial y preservando mejor los compuestos bioactivos los tratamientos a menor temperatura. Además, el uso malla impregnada con alfacipermetrina resultó ser eficaz en el control físico y químico de las principales plagas de almacén en estado larvario y así evitar reinfestaciones durante el almacenamiento. Por lo tanto, la implantación de estos sistemas de control de agentes bióticos alterantes podría contribuir sustancialmente a mejorar la calidad higiénico-sanitaria del higo seco en Extremadura.

ABSTRACT

ABSTRACT

The objective of this study was to analyze the biotic agents affecting dried figs in Extremadura, as well as to evaluate the effectiveness of control systems during the production process to minimize the damage caused by them. For this purpose, samples of dried figs were taken from 18 fig orchards with different levels of management, with birds being the leading cause of damage, followed by insects (*Cadra figulilella*, *Carpophilus hemipterus* and *Ceratitis capitata*) and moulds (*Aspergillus*). In addition, in irrigated fig orchards, agricultural management takes on greater importance for pest control. In this context, in the field during fruit ripening and drying, the use of the netting system adapted to the canopy significantly reduced damage caused by birds and insects of the order Lepidoptera, as well as mycotoxin contamination, while also improving physicochemical characteristics: fruit weight, colour and firmness. In the industry, freezing treatments (-18ºC 1 day, -10ºC 2 days or -5ºC 7 days) proved an effective alternative to phosphine in insect control, maintaining sensory quality and better preserving bioactive compounds at lower temperatures. In addition, using alphacypermethrin-impregnated netting proved effective in the physical and chemical control of the primary storage pests in the larval stage, thus avoiding reinfestations during storage. Therefore, implementing these systems to control altering biotic agents could contribute substantially to improving the hygienic-sanitary quality of dried figs in Extremadura.

INTRODUCCIÓN

INTRODUCCIÓN

1. Cultivo de la higuera

La higuera (*Ficus carica* L. 2n=26), domesticada desde hace más de 7000 años en la cuenca mediterránea y Asia occidental, es una de las especies frutales asociadas al nacimiento de la fruticultura (Langgutt and Garfinkel, 2022). El género *Ficus*, con 850 especies extendidas por los trópicos y subtrópicos de todo el mundo, es el más importante de los 40 géneros que integran la familia Moraceae (Aksoy and Flaishman, 2022).

Es un árbol o arbusto caducifolio y su porte puede variar de esparcido a erecto dependiendo de la variedad (Morton, 1987). El tamaño de la copa y la densidad de ramificación dependen del genotipo, si bien, factores como el manejo y calidad del suelo pueden influir considerablemente en su desarrollo. Existen ejemplares excepcionales que llegan a 10 m de diámetro y a 9 -12 metros de altura, pero normalmente las plantaciones comerciales albergan arboles pequeños (Flaishman, 2022).

El sistema de formación más utilizado en el cultivo de la higuera es el vaso, si bien la altura de formación de las ramas principales depende del destino de los frutos. Para la producción de higos secos se requiere mayor espacio debajo de la copa del árbol, siendo esta altura de formación de ramas en torno a 1 m, que facilita el secado y recogida de los higos en el suelo o sobre mallas (Figura 1). Por el contrario, para la producción de higo fresco la altura de formación se reduce a los 50cm ya que la recolección de frutos se realiza desde el suelo (Jafari et al., 2022).

Figura 1. Porte de una higuera (*Ficus Carica* L.) destinada a la producción de higo seco. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

El sistema radicular de la higuera es fibroso, superficial y sin raíz pivotante, extendiéndose hasta tres veces el diámetro de la copa (Condit, 1947). El tronco es de madera blanda compacta y cuenta con una corteza gris, suave y sin fisuras (Crisosto et al., 2011). En el tronco y las ramas de más de dos años pueden desarrollarse hinchazones nodales a ambos lados o por debajo de las cicatrices peciolares. Las ramas primarias son lisas, glabras, de color grisáceo y no muy numerosas. Sin embargo, el número de ramas secundarias es variable, son de madera de poca densidad y de engrosamiento anual visible (Costa, 2019). En las ramas se localizan las yemas o botones, que pueden ser de flor y de madera. Ambas son axilares y se sitúan por encima de la inserción del peciolo. Al final de cada rama se sitúa una yema apical, desde la cual se inicia el crecimiento anual de la rama. En la base de las ramas o del tronco se sitúan las yemas adventicias, dando origen a rebrotes en circunstancias favorables. Las hojas de higuera son pecioladas y palmeadas, de tamaño grande, color verde brillante, simples y alternas. Pueden tener de tres a cinco lóbulos, llegando en algunos casos hasta siete (Gaaliche, 2022).

En relación con la explotación de la higuera, hay que mencionar que se trata de una especie dioica con dos morfologías diferenciadas, el árbol masculino o cabrahigo y el árbol femenino o común destinado para la producción de frutos comestibles. Basándonos en su compleja biología floral la higuera femenina puede dividirse en tres tipos. De tipo común, no requieren polinización (partenocárpicas) y divididas a su vez en: uníferas, solo producen una cosecha de higos en la madera del año; y bíferas, producen dos cosechas, una de brevas en la madera del año anterior y otra de higos en la madera del año. Por otro lado, las de tipo San Pedro, son bíferas, presentan una primera cosecha de brevas sin necesidad de polinización y requieren polinización para la producción de higos. Por último, las de tipo Esmirna son uníferas y necesitan ser polinizadas para desarrollar los higos (Storey, 1976; Lopéz-Corrales et al., 2012). La polinización la realiza de forma pasiva una especie de himenóptero, *Blastophaga psenes L.* y este proceso se conoce como caprificación.

Las flores y los frutos se encuentran localizados en el interior de un receptáculo carnoso de forma redondeada denominado sicono. En la Figura 2 se muestra gráficamente las diferentes partes que componen un higo. Este receptáculo contiene numerosas flores de tamaño muy pequeño y orientadas hacia su centro; las flores estaminadas sólo están presentes en el cabrahigo y las flores pistiladas de estilo corto o brevistilas y de estilo largo o longistilas. Cuando maduran dan lugar a unos frutos secos denominados aquenios y a un periantio carnoso y dulce que va a dar lugar a que el receptáculo se vuelva engrosado y sabroso, que es lo conocemos como higo o breva (López -Corrales et al., 2012). Por tanto, lo que comúnmente se denominan frutos (brevas e higos) son considerados como “falsos frutos” (Lisci y Pacini, 1994). Las brevas, primera cosecha en la madera del año anterior, y los higos, segunda cosecha en la madera nueva. En cuanto a la forma puede ser turbinado, ovoide, piriforme, cucubiforme o urceolado y la piel puede

variar de verde amarillento a púrpura, rojizo o bicolores. El ostiolo es la principal vía de entrada de insectos y patógenos (Kong et al., 2013), por lo que su tamaño es muy tenido en cuenta a la hora de selección de variedades comerciales.

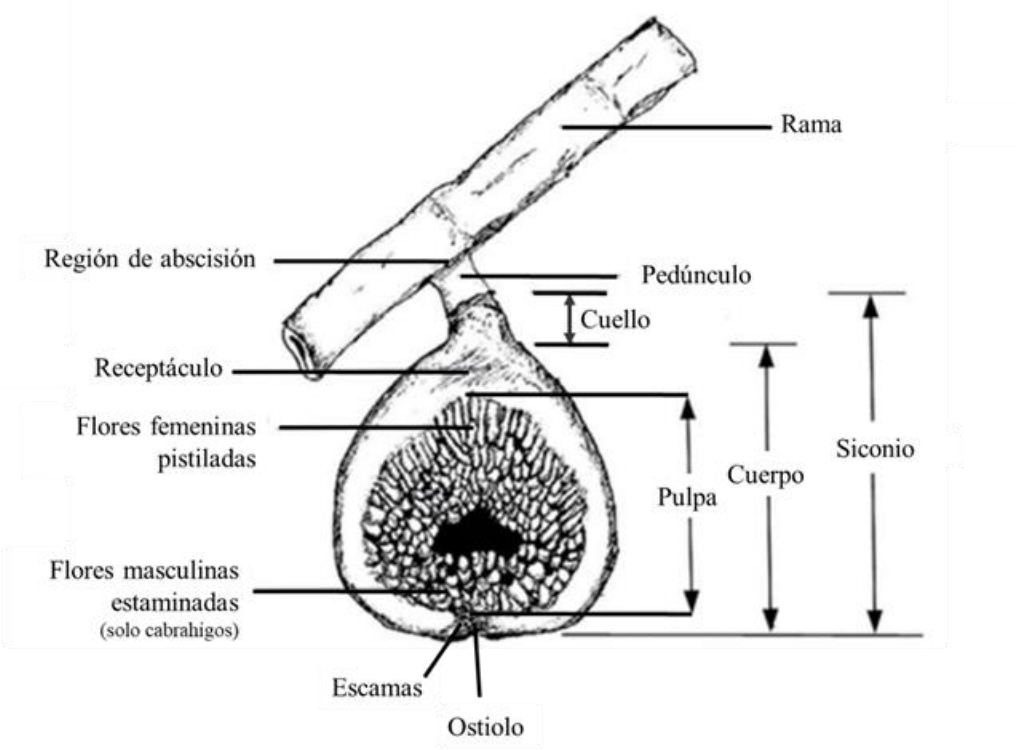


Figura 2. Partes del higo. Fuente: López- Corrales et al., 2012.

2. Importancia socioeconómica del cultivo de la higuera

La superficie estimada mundial cultivada de higueras en el año 2021 fue de en torno a 299.540 ha, obteniendo una producción total de 1.348.254 t (FAOSTAT, 2021). Como muestra la Figura 3, el ranking de producción mundial lo lidera Turquía con 320.000 t, seguido de Egipto, Marruecos, Argelia e Irán. España ocupa el sexto lugar con 60.190 t (FAOSTAT, 2021).

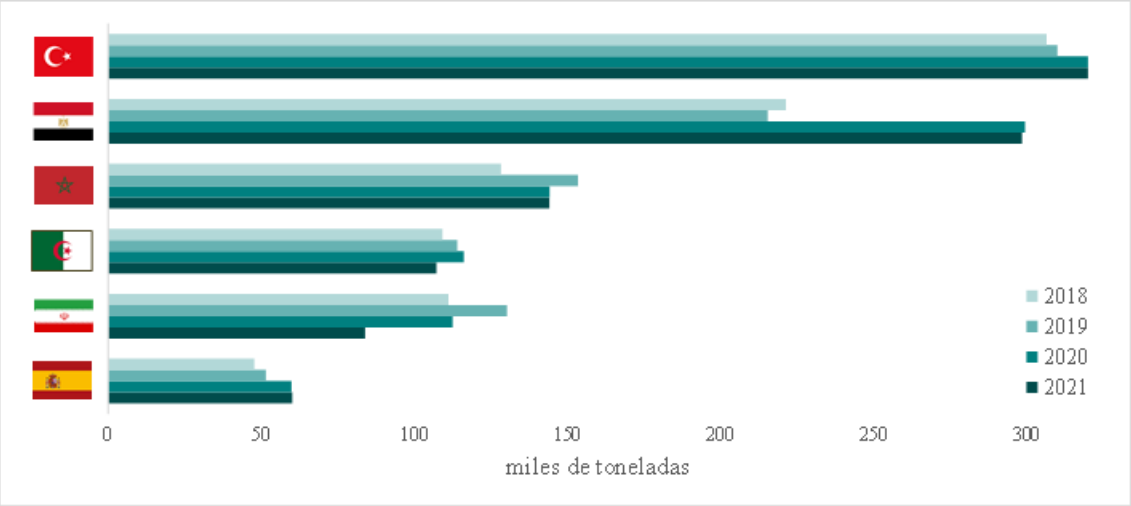


Figura 3. Producción anual de los principales países productores de higo. Fuente: FAOSTAT, 2021.

Considerando que el ratio de higo fresco/ seco es 4:1 aproximadamente, se puede considerar que el 50% de la producción total de higo es destinada a secado (520 – 640 mil toneladas anuales) y su posterior venta como higos secos (Tırkaz et al., 2022). Con una producción total de 142.500 t en la campaña 21/22, el higo seco ocupó el 5% de la producción mundial de frutos secos. El primer país productor de higos seco es Turquía con un 53% de la producción, seguido de Irán y España con 18 y 8%, respectivamente (Figura 4; INC, 2022).

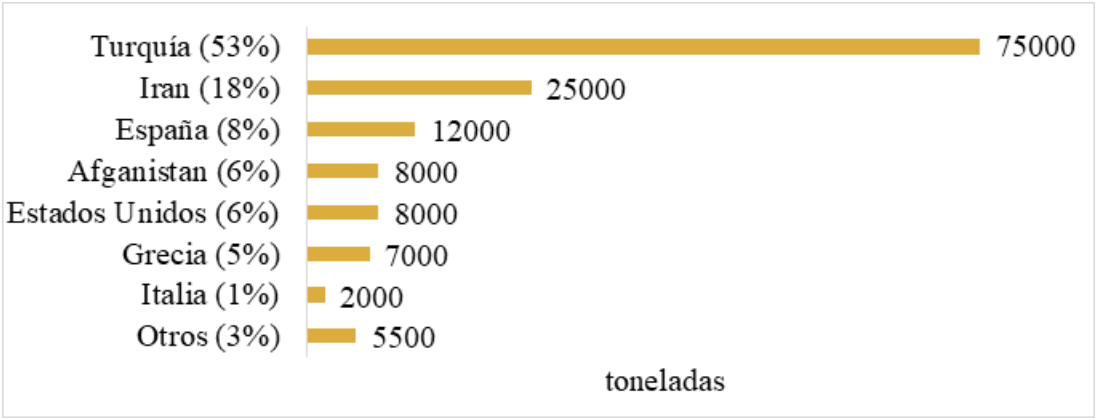


Figura 4. Producción de higo seco a nivel mundial durante la temporada 20/21. Fuente: INC, 2022.

Hay que destacar que España es el principal país de la Unión Europea en producción de higo, con una superficie cercana a las 17.000 ha. Más del 64% de la

producción española se concentra en la Comunidad Autónoma de Extremadura, que en 2021 produjo 38.621 t en una superficie de 7904 ha (Figura 5). El resto se distribuye por toda la península ibérica destacando, Cataluña (6,7%), Comunidad Valenciana (5,8%), Galicia (4,8%), Andalucía (4,6%) y Castilla la Mancha (3,9%) (MAPA, 2021).

El espectro varietal español de higuera está compuesto por variedades locales, resultado de la selección natural promovida por las condiciones edafoclimáticas y por una selección artificial por la acción del agricultor (López-Corrales et al., 2016). Las principales variedades cultivadas en la zona este del país son Coll de Dama Negra, Albacor y Burjassot Negra (Figura 5). La variedad Cuello Dama Blanco, probablemente una de las más cultivadas en el mundo, se extiende por la zona de Extremadura, Castilla León y Castilla la Mancha. En cuanto a 'Calabacita' y 'San Antonio' son de las más extendidas en la mitad sur de Extremadura para seco y fresco respectivamente. En Andalucía se cultivan diferentes variedades locales, entre ellas se encuentra 'Blanca de Pasa', localizada en la Sierra granadina de la Contraviesa, y 'Negra Común', en la zona de Huelva.

Figura 5. Producción española de higo por comunidades autónomas y principales variedades cultivadas. Fuente: MAPA, 2021.

Tradicionalmente en Extremadura la higuera ha sido considerada un cultivo marginal, establecida en plantaciones de secano, con amplios marcos de plantación y con mínimos cuidados culturales. Los frutos eran destinados a la alimentación humana y animal, principalmente ganado porcino (López-Corrales et al., 2012).

En los últimos años, la higuera ha adquirido gran relevancia en la región. Por un lado, la crisis que ha sufrido el sector de la fruta de hueso ha llevado a los fruticultores a la diversificación con la búsqueda de cultivos alternativos. Por otro, el consumo de higos ha aumentado recientemente, siendo cada vez más demandado en los mercados nacionales e internacionales, lo que ha causado un incremento en los precios de venta (Shokoohi et al., 2022).

Este auge ha propiciado un mayor interés del sector por el cultivo, por ello, se han incorporado progresivamente nuevas técnicas de manejo que permiten obtener un

producto de mayor calidad y más rentable. Como muestra la Figura 6, en Extremadura establecimiento de nuevas plantaciones de higuera en regadío comenzó en el año 2015, alcanzando en 2021 las 1.385 ha. Las consecuencias de este nuevo panorama se han visto reflejadas en el aumento del rendimiento del cultivo en los últimos diez años. Entre el periodo 2012- 2021 el incremento en la producción fue de más del 600%, sin embargo, la superficie solo se vio incrementada en un 150%.

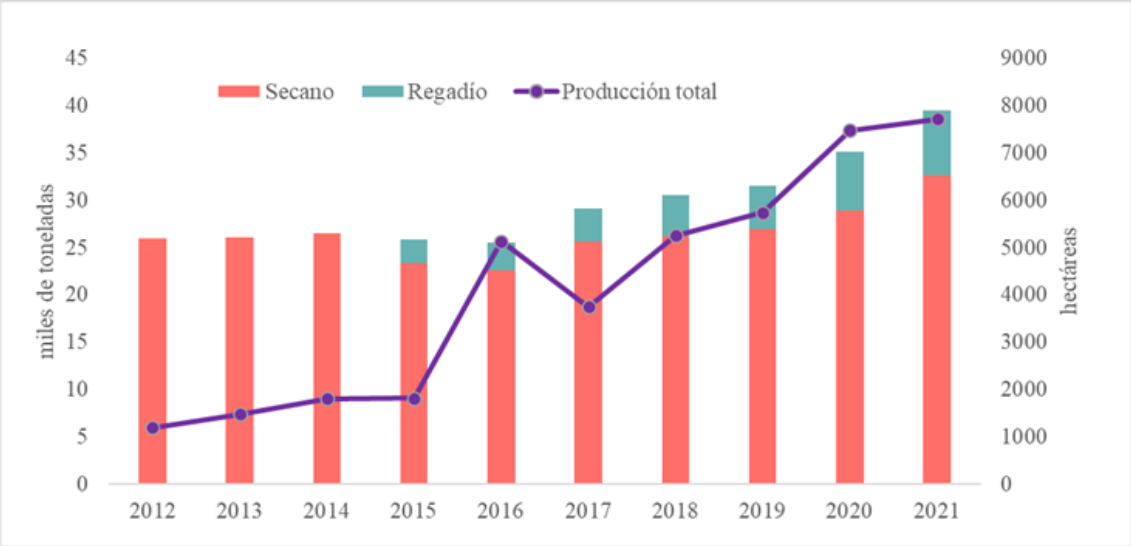


Figura 6. Evolución de la producción y de la superficie cultivada (secano/regadío) de higuera entre 2012 y 2021. Fuente: MAPA, 2022.

En relación al comercio exterior, en 2021 en el mundo se exportaron un total de 204.232 t entre higo fresco y seco (FAO, 2021). Este producto fue comercializado por un total de 772 millones de dólares, siendo los principales países exportadores Turquía (43,8%), Afganistán (21,2%), Austria (4,44%), España (3,59%) y Alemania (3,37%) (OEC, 2021). España exportó un total de 2.448 t de higo seco y 5.485 t de fresco por un valor de 7,5 y 13,7 millones de euros respectivamente (Datacomex, 2021). Los principales países importadores de higos, para consumo o comercializarlos, fueron India (20,7%), Alemania (14,7%), Francia (8,29%), Estados Unidos (6,18%) y Países Bajos (4,23%) (OEC, 2021). En cuanto a España, importó 1.584 t por un valor de 5,2 millones de euros (Datacomex, 2021).

3. El higo seco

La maduración de los higos es heterogénea, encontrándose frutos en diferentes estados fisiológicos en un mismo ramillete (Figura 7; Şen, 2022). Estos higos completamente maduros permanecen en la rama mientras se produce su senescencia, comienzan a perder agua y el pedúnculo se separa de la rama. Por esta razón, desde hace miles de años los higos terminan su secado de forma natural al sol (Şen, 2022) que reduce la de humedad y a_w de los frutos. Todo ello disminuye la actividad microbiana y limita los cambios físicos-químicos, proporcionando durabilidad, resistencia al deterioro y manteniendo la calidad del producto. Este método es el más extendido para preservar la calidad de los higos (Farahnaky et al., 2009; Mujić et al., 2014; Kutlu et al., 2015).

Figura 7. Higos en diferentes estados de sobremaduración. Fuente: Dpto. Fruticultura Mediterránea (CICYTEX).

En la actualidad existen dos métodos básicos para deshidratar los frutos: secado al sol, utilizando el calor del sol en condiciones naturales, o secado artificial, utilizando sistemas artificiales de generación de calor (Mat Desa et al., 2019). Debido al bajo coste de producción e inversión, unido a que en la mayoría de las regiones productoras de higo seco en el momento de cosecha las condiciones ambientales son áridas y cálidas, el sistema más extendido es el secado natural al sol (Şen, 2022). Este secado se realiza dejando los higos secando en el árbol y cosechándolos una vez caídos al suelo o recolectando los frutos ya madurados del árbol y disponiéndolos en bandejas para terminar el secado (Figura 8; Haug et al., 2013).

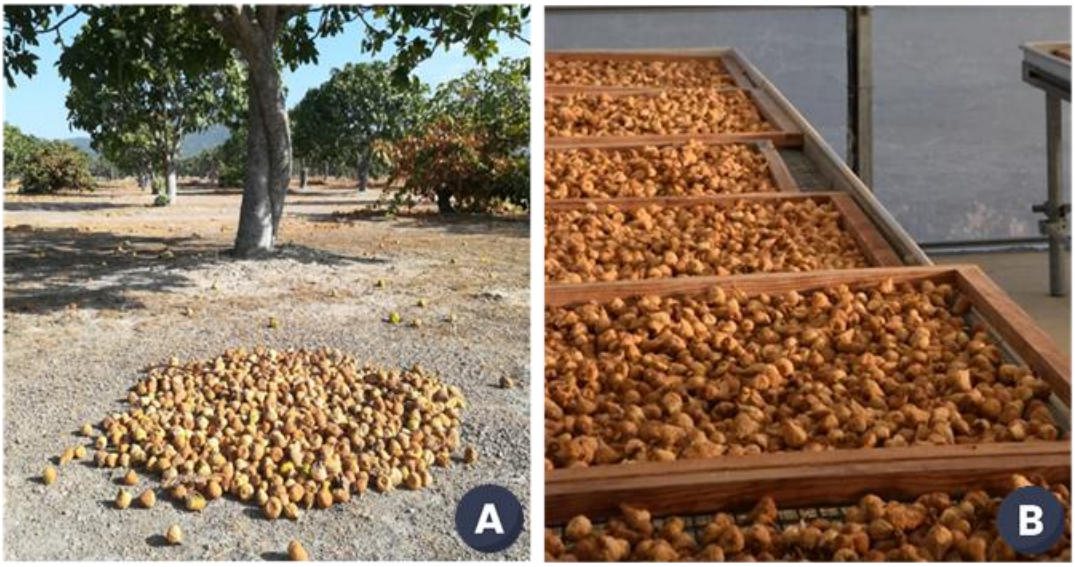


Figura 8. a) Secado solar en el suelo del higueral y b) Secado solar en paseras. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

Durante el secado natural las condiciones higiénicas- sanitarias no son controladas completamente ya que los frutos permanecen al aire libre y están expuestos a una amplia diversidad de agentes alterantes (Belessiotis y Delyannis, 2011; Chand et al., 2015). En los sistemas de secado artificial el proceso de secado se realiza bajo condiciones controladas, en un periodo de tiempo más corto, garantizando en parte las condiciones de calidad sensorial e higiénicas (Slatnar et al., 2011). Sin embargo, estos sistemas requieren altas inversiones y costes de funcionamiento, además de la connotación negativa de las

emisiones de carbono que pueden producir al medio ambiente (Şen, 2009). En los últimos años, para solventar gran parte de los problemas higiénicos- sanitarios años se han llevado a cabo numerosos estudios relacionados con diferentes sistemas de secado artificial de higo. Entre ellos, se encuentra el uso de cabinas de secado (Piga et al., 2004; Xanthopoulos et al., 2007, 2010; Tan, 2017), estufas (Villalobos et al., 2016), túneles de secado (Babalis and Belessiotis, 2004; Babalis et al., 2006) o microondas (Sharifian et al., 2012; Abul-Fadl et al., 2015). Algunos de estos sistemas basados en secado mediante aire caliente ya se emplean en países productores como Turquía (Villalobos, 2015).

En cuanto a características nutricionales, el higo es un componente importante en la dieta mediterránea, siendo considerado símbolo de longevidad (Çalişkan and Polat, 2011; Arvaniti et al., 2019). El higo seco destaca por su alto contenido en hidratos de carbono, principalmente fructosa y glucosa, encontrándose entre los frutos secos más nutritivos y energéticos (Melgarejo et al., 2003; Veberic and Mikulic-Petkovsek, 2016). También es rico en fibra y minerales, entre los cuales destaca el potasio, seguido de calcio y sodio. La vitamina C y la vitamina K son las principales, mientras que en cuanto a ácidos orgánicos destacan el ácido cítrico y el ácido málico (Serradilla et al., 2022). Estos frutos tienen bajo contenido en grasas y están libres de sodio y colesterol (Vinson, 1999; Solomon et al., 2006; Çalişkan, 2015). Además del valor nutritivo, la fruta también contiene compuestos no nutritivos o fitoquímicos bioactivos. Los principales en higos son los flavonoides, cumarinas y compuestos volátiles (Pereira et al., 2017; Yang et al., 2023) En higos secos el contenido nutricional, en relación a compuestos fenólicos y actividad antioxidante es mayor que en higos frescos (Slatnar et al., 2011; Şen, et al., 2017), debido a la concentración de los compuestos tras la pérdida de humedad. La composición de los higos secos depende en gran parte del genotipo, manejo del cultivo, estado de maduración

en la cosecha, proceso de secado y condiciones de almacenamiento (Crisosto et al., 2010; Çalişkan and Polat, 2011; Slatnar et al., 2011).

Es considerado un alimento funcional y cuenta con diversas propiedades beneficiosas para la salud, como actividad antioxidante, antidiabética, anticancerígena, neuroprotectora, antiinflamatoria y antiviral (Purnamasari et al., 2019; Ayuso et al., 2022), lo que ha generado un gran interés en la sociedad. Además de los anteriores, la aceptación del consumidor viene determinada por otros parámetros de calidad, siendo la humedad, el tamaño, el color y la textura los principales para higo seco (Figura 9).

Figura 9. Higos secos de alta calidad. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

Asimismo, también son importantes la forma del producto, atractivo visual, sabor, retención de nutrientes, propiedades de rehidratación, a_w, estabilidad química, carga microbiológica, ausencia de insectos, plagas y contaminantes y libres de malos olores (Perera, 2005). Algunos de los parámetros están regulados por la normativa de la UNECE (Comisión Económica de las Naciones Unidas por Europa) DDP-14, en la que se recogen los estándares de comercialización y control de calidad comercial de higos secos (Tabla 1; UNECE, 2016).

Tabla 1. Tolerancias de calidad de la guía STANDARD DDP-14 (UNECE, 2016).

	Tolerancias permitidas, porcentaje de productos defectuosos, por número o peso		
Defectos permitidos	*Extra*	*Clase I*	*Clase II*
(a) Tolerancias para los productos que no cumplan los requisitos mínimos de los cuales no más de:	10	15	30
Dañados por plagas	9	12	15
Seriamente dañados por el sol, partidos o excesivamente seco	8	10	20
Mohoso y Fermentado	3	4	5
Plagas vivas	0	0	0
(b) Tolerancias de tamaño			
Los productos que no se ajusten al calibre indicado en total	20	20	20
(c) Tolerancias para otros defectos			
Materias extrañas, tallos sueltos, ostiolo y polvo (en peso)	0,5	0,5	0,5
Entre las variedades de higos blancos (de color blanco a marrón oscuro de color) y entre las variedades de higos negros (de color púrpura a negro), los higos secos que son claramente diferentes en color, por recuento	10	10	10

4. El higo seco en Extremadura

Tradicionalmente, en Extremadura y en el resto de la Península Ibérica el manejo del cultivo de la higuera ha sido muy tradicional. Un gran número de higueras diseminadas por todo el territorio, se han establecido en plantaciones de secano con amplios marcos de plantación (10 x 10 m). Este sistema, se ha caracterizado por sus mínimas prácticas culturales: sin poda y sin aplicación de fitosanitarios ni fertilizantes y solo se aplicaba estiércol en el momento de establecer el cultivo (López-Corrales et al., 2012). Como resultado, se obtenían árboles altos con grandes copas, pero con rendimientos normalmente bajos (1500 kg/ha) (López-Corrales and Balas, 2014). El aumento en la demanda y de los costes de producción del higo seco ha impulsado la

búsqueda y el establecimiento de la higuera en sistemas de mayor eficiencia productiva más rentables y sostenible (Galván et al., 2021).

En los últimos años, se han estudiado nuevos sistemas de formación en regadío intensificando los marcos de plantación con el objetivo de determinar el comportamiento agronómico de distintas variedades y la calidad de los frutos. En cuanto a higo fresco, Pereira et al., (2017) estudió el comportamiento de 10 variedades de higuera en Extremadura, formadas en vaso, en marco intensivo (5 x 4 m) y con riego localizado, obteniéndose rendimientos tres veces superiores a los a los de secano. En el caso del higo para seco, Galván et al., (2019) estudió el comportamiento de las tres variedades de higuera más cultivadas para seco en Extremadura (Calabacita, Cuello Dama Blanco y Picholetera) formadas en vaso alto y superintensivo (1000 árboles/ha), con riego localizado. Para la recolección de los higos, se implementó un sistema de mallas suspendido a 50 cm del suelo (Figura 10), obteniéndose producciones en torno a las 10 t/ha al noveno año de plantación, además de una mejora higiénico- sanitaria de los higos.

Figura 10. Recolección con mallas suspendidas en el aire en plantación superintensiva. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

Del mismo modo, diferentes estudios sobre la influencia de la poda, régimen hídrico (Micheloud et al., 2018; Abdolahipour et al., 2019; Zare, 2021) y fertilización

(İrget et al., 2008; Osman and Abd El-Rhman, 2010; Tofanelli et al., 2022) han sido llevado a cabo en otras zonas productivas del mundo. Abdolahipour et al. (2019), en condiciones de secano, observaron que el aclareo del tronco junto con la poda de rama (eliminando ~75% de madera) tuvo efectos positivos en los parámetros de tolerancia de la planta a la sequía, mejorando también las características de los frutos. Respecto a la fertilización, Tofanelli et al. 2022 concluyó que la aplicación de 5 L de estiércol bovino por higuera, en 4 aplicaciones a lo largo del año, aumenta el número de frutos por planta, incrementando también el peso, longitud y diámetro de los mismos, en comparación con las plantas sin fertilizar.

De forma general, la tendencia de las nuevas plantaciones de higo seco en Extremadura es a establecer los árboles en marcos de plantación intensivo (5 x 4 m), con árboles de tamaño más pequeño, riego localizado con gotero en el centro de las calles, poda anual y fertilización de invierno (NPK) y de primavera (nitrato de potasio).

Como se indicó anteriormente, Extremadura lidera la producción de higo a nivel nacional con una superficie en 2021 de 20.697 y 17.924 ha en Badajoz y Cáceres, respectivamente (Figura 11; MAPA, 2021).

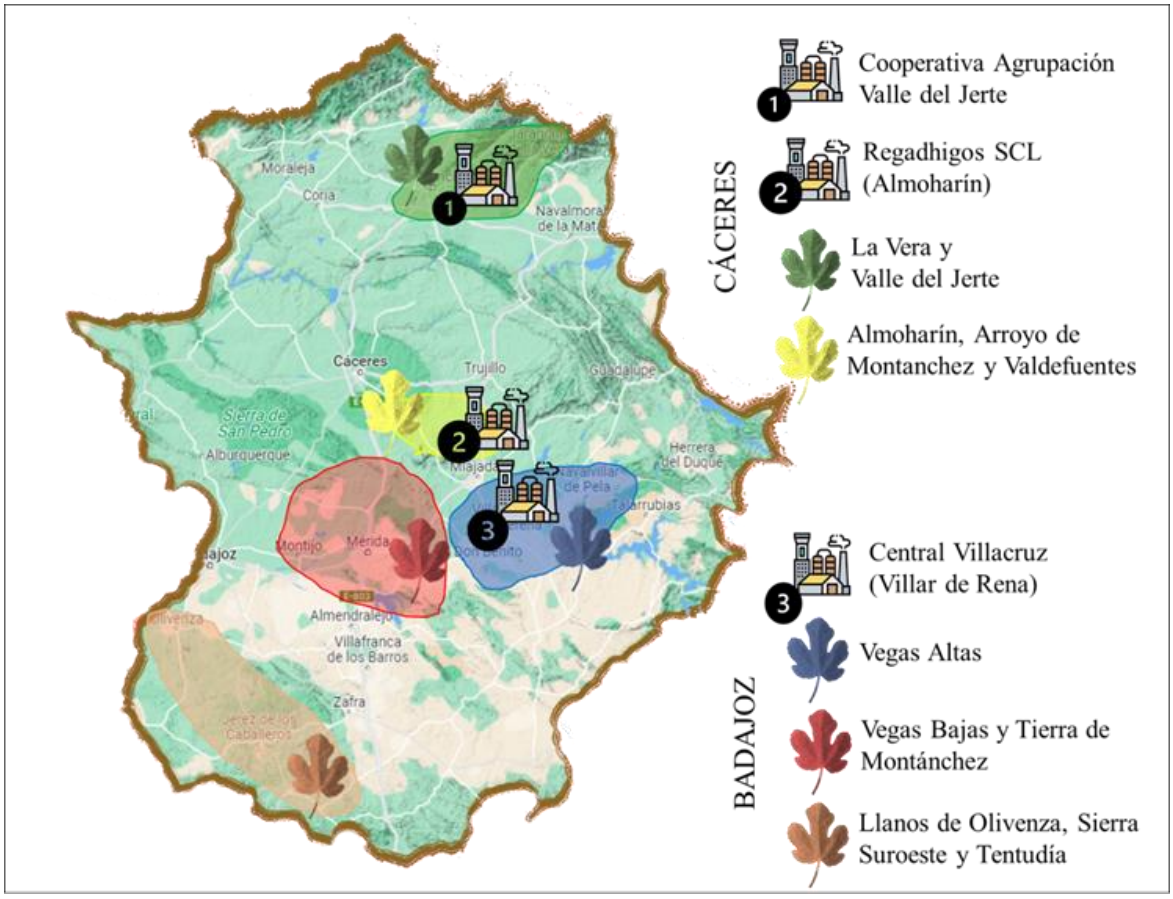


Figura 11. Mapa con principales zonas de producción de higo seco e industrias transformadoras. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

En la provincia de Badajoz los higuerales se localizan al sur, en las comarcas de Tentudía, los Llanos de Olivenza y Sierra Suroeste, y al norte, en localidades como La Nava de Santiago, La Roca o Trujillanos pertenecientes a la comarca de las Vegas Bajas y Guareña en las Vegas Altas (Figura 11). En la provincia de Cáceres, se diferencian dos zonas productoras, al sur, en los municipios de Almoharín, Arroyomolinos de Montánchez o Valdefuentes en la comarca Tierra de Montánchez, y al norte de la provincia, en las comarcas de La Vera y del Valle del Jerte (López-Corrales et al., 2012).

Las principales industrias de procesado de higo seco en Extremadura son: la Agrupación de Cooperativas Valle del Jerte, Regadhigos y central hortofrutícola Villacruz, localizadas en Valdastillas, Almoharín y Villar de Rena, respectivamente (Figura 11).

En relación al material vegetal en Extremadura, se encuentra el Banco de Germoplasma de higuera, localizado en el Centro de Investigaciones Científicas y Tecnológicas de Extremadura, CICYTEX- "La Orden" en Guadajira (Badajoz; Figura 12). En la actualidad, en este banco se conservan más de 350 variedades distintas, de ellas un 85% son representativas de las cultivadas en España destinadas a producir tanto para consumo en fresco, como para seco.

Figura 12. Banco de germoplasma de higuera de CICYTEX, Guadajira (Badajoz).

Este centro cuenta con 46 entradas de variedades locales de la zona de Extremadura, perteneciendo en su mayor parte al grupo de partenocárpicas (bíferas o uníferas) (López-Corrales et al., 2016). De todas ellas, una decena son las más cultivadas en la región. Para la producción en fresco destacan las variedades Albacor, De Rey, Tiberio, Negra Cabezuela, San Antonio, Nazaret y Dalmatie, mientras que para la producción de higo seco las más cultivadas son Calabacita, la Casta y Cuello Dama Blanco, está ultima con doble aptitud (López-Corrales et al., 2012; López-Corrales et al., 2016).

La variedad **Calabacita**, originaria de Extremadura, es la más extendida para producir higo seco debido a su elevada calidad organoléptica (Figura 13; López-Corrales et al., 2012). Mayoritariamente establecida en las comarcas de Vegas altas, Vegas Bajas y la Sierra de Montánchez. Es una variedad bífera, con baja producción de brevas, pero alta de higos y adaptada tanto a secano como a regadío. Los frutos tienen un tamaño entre 35 y 45 mm de diámetro, forma esférica, piel de color amarillo verdoso y pulpa de color ámbar (López-Corrales and Balas, 2016). Es una variedad temprana, por lo que la recolección de higos secos abarca desde finales de julio hasta mediados de septiembre, situándose el pico de máxima producción a mediados de agosto (Galván, 2022).

Figura 13: Higos secos de la variedad Calabacita.

Otra variedad relevante para secado es **Cuello Dama Blanco (Figura 14)**. Tiene un comportamiento óptimo en las condiciones climáticas del norte de Cáceres, por lo que principalmente se cultiva en la comarca del Valle del Jerte y La Vera. Es una variedad bífera, con reducida producción de brevas y muy productiva de higos. Los frutos son de tamaño grande entre 50 y 55 mm de diámetro, por ello también se destinan a consumo en fresco. Tienen forma esférica, piel gruesa, elástica y resistente, de color verde amarillento y pulpa ámbar. Su fecha de maduración es media, por lo que las últimas recolecciones pueden verse afectadas por las primeras lluvias de otoño (López-Corrales et al., 2012).

Figura 14. Higos secos de la variedad Cuello Dama Blanco.

Al sur de la provincia de Badajoz está presente otra variedad, La Casta (Figura 15), la cual es una variedad en recesión que ha sido muy cultivada. Es unífera y con fecha

de maduración media. Los frutos son esféricos de tamaño medio, piel fina de color verde amarillento y pulpa roja anaranjada. Debido a su baja calidad organoléptica sus frutos son normalmente son destinados a pasta de higo o alimentación animal (López-Corrales et al., 2012).

Figura 15. Higo seco de la variedad Casta

Además, en la zona de norte de Cáceres dos variedades muy extendidas son Picholetera, característica por su cuello largo, piel elástica y doble aptitud, y Granito, de producción de higos temprana y esféricos (Lopéz-Corrales et al., 2012).

5. Proceso productivo

En la Figura 16 se muestra de forma esquemática el diagrama de flujo del proceso de producción del higo seco tanto en su etapa precosecha (campo) como postcosecha (Industria). A fin de garantizar la producción de higos secos con excelente calidad organoléptica e higiénico- sanitaria, es fundamental controlar la calidad del producto en cada una de las etapas de producción, desde el campo, pasando por la recolección, el secado, el almacenamiento y el transporte. Este proceso no es idéntico entre zonas productivas, ni entre industrias, ya que existen variaciones en las técnicas utilizadas en las diferentes etapas, en los periodos de tiempo o en la temperatura de conservación (Galván, 2022).

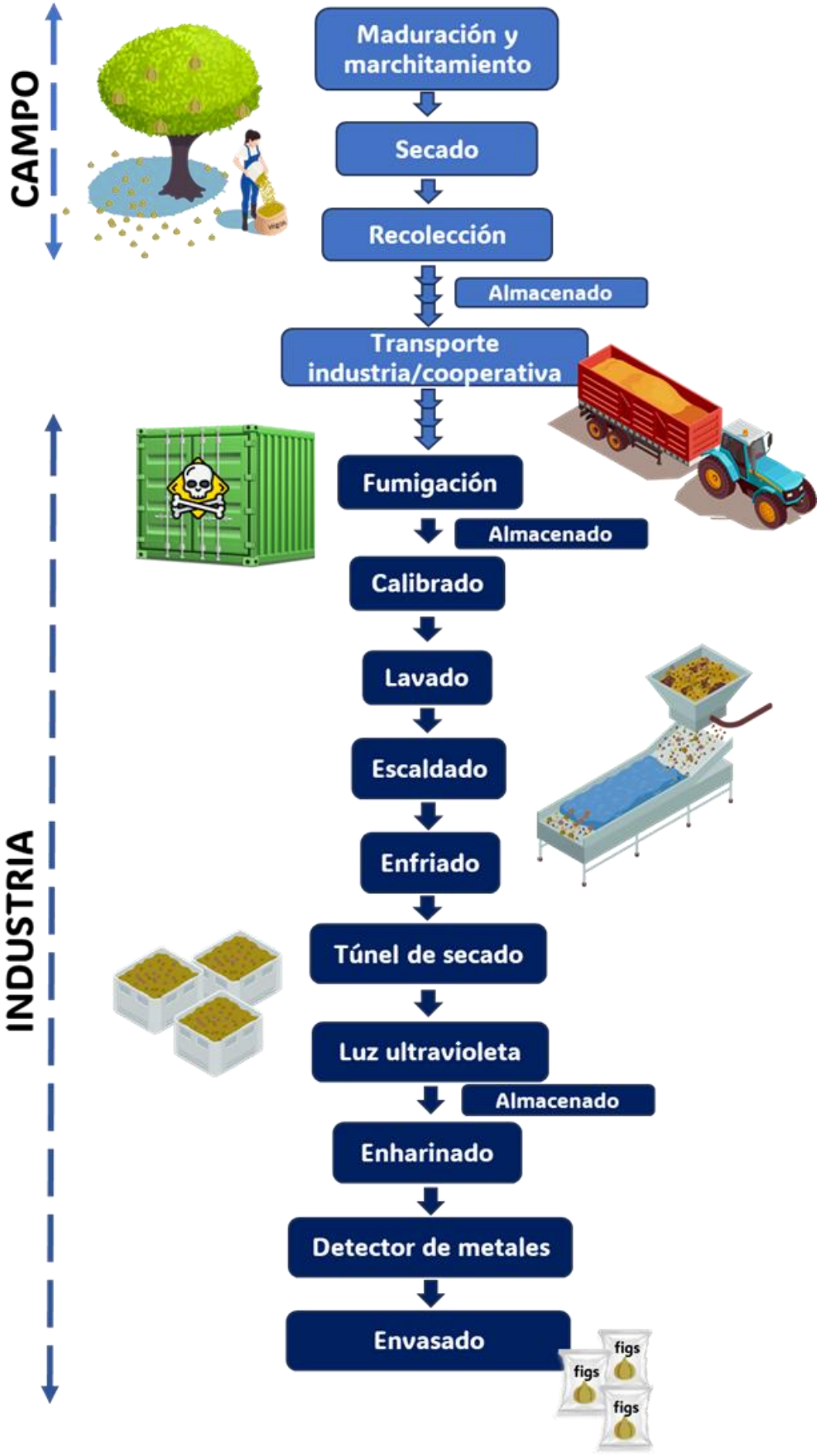


Figura 16. Diagrama de flujo del proceso de producción de higo seco.

La etapa de maduración, secado y recolección del higo se da entre los meses de agosto y septiembre. A diferencia de aquellos destinados al consumo en fresco, los frutos que serán deshidratados no se recolectan cuando alcanzan la madurez comercial. En su lugar, permanecen en las ramas de la higuera, donde comienzan a marchitarse. El contenido de humedad de los frutos en la fase inicial de marchitamiento se sitúa alrededor de 69-73% dependiendo de la variedad (Şen et al., 2017). Tras 5-6 días de madurar, dependiendo de la variedad y las condiciones de temperatura y humedad relativa (HR) del ambiente, los higos comienzan a caer al suelo de forma natural (Figura 17). En esta etapa, los higos todavía no han adquirido una a_w que nos garantice un producto seguro, presentando una humedad de alrededor del 30-50% (Aksoy 1997; Şen et al., 2017). Como se ha indicado previamente, existen varios métodos para el secado de los frutos, siendo el más extendido el secado natural al sol. Este puede llevarse a cabo completamente en el suelo, en climas cálidos y áridos (40ºC/ 40-50% H.R), o recolectando los frutos y extendiéndolos en paseras ubicadas en invernaderos o almacenes para completar la deshidratación (Figura 16). El secado finaliza cuando el contenido de humedad de los higos es inferior al 26% de acuerdo con la norma de calidad DDP-14 relativa a la comercialización y control de calidad de los higos secos (UNECE, 2016). La recolección se lleva a cabo periódicamente, aproximadamente cada 1 o 2 semanas (Figura 18); se realiza de forma manual, dejando en el campo los higos con daños visibles (pájaros,

Figura 17. Higos secados en el suelo.

mecánicos, hongos…), o mediante sopladoras o rastrillos, realizando la limpieza y clasificación posteriormente.

Figura 18. a) Recolección de higo seco con sopladora, b) recolección manual de higo seco. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

La maduración y secado es un proceso gradual, por lo que el periodo desde que el agricultor comienza a recolectar higos hasta alcanzar un determinado volumen para comercializarlo puede prolongarse. Por esta razón, algunos agricultores necesitan almacenar durante algún tiempo los higos. La limpieza y el control de las condiciones ambientales de las instalaciones son fundamentales para mantener la seguridad y calidad del producto (Şen, 2009). Además, entregar los higos a las industrias lo más rápidamente posible, reducirá el riesgo de infestación de plagas de almacenamiento.

Los higos, almacenados en palots, son transportados hasta las industrias/ cooperativas donde son procesados. Antes de ser procesados, los higos son sometidos a una desinfestación en las instalaciones de la industria para eliminar las plagas procedentes del campo. Desde la prohibición del uso del bromuro de metilo (Şen et al., 2010), el método más extendido es la fumigación con fosfuro de aluminio y/o magnesio. Para ello, se introducen los palots con higos secos bajo plástico impermeable o en un contenedor sellado, a temperatura ambiente, durante 5 días (Figura 19). El fosfuro de aluminio o magnesio, aplicado en dosis de 5-10 g PH_3/m^3, reacciona con la humedad produciendo fosfuro de hidrógeno, denominado fosfina, letal para la mayoría de las plagas del higo seco. Tras esto, los frutos se almacenan a temperatura ambiente o en el mejor de los casos en cámara frigorífica, donde el desarrollo de microorganismos e insectos está controlado, hasta ser procesados en la industria (Figura 20). La humedad ideal en higos secos para su almacenamiento es 18-22%, preferiblemente inferior a 24% y una a_w menor de 0,65 (Okşar, 2017).

Figura 19. Fumigación bajo plástico impermeable.


Figura 20. Higos secos en palots almacenados en la industria. Fuente: www.regadhigos.es.

La clasificación de los frutos se realiza de forma mecánica (Figura 21), mediante tamices de diferentes diámetros. La norma DDP-14 (UNECE, 2016) hace opcional el calibrado bien en función del número de frutos por kg o bien en función del diámetro. En el caso de número de frutos, va desde el calibre 1 con 40 frutos hasta el calibre 11 con más de 120 higos secos por kg. Si se clasifica por tamaño, para higo claro, debe tener un mínimo de 22 mm. Existen normas estadounidenses (USDA, 2021) y turca (TS-541) que, del mismo modo, regulan la calidad de los higos secos en los diferentes países. Durante esta etapa se eliminan objetos extraños como piedras, plásticos, palos o tierra entre otros. Actualmente, algunas industrias han incorporado equipos de rayos X que detectan y retiran estos materiales. Una vez clasificados los higos secos son transportados a través de cintas mecánicas al proceso de lavado.


Figura 21. Clasificación de frutos por categorías.

Los frutos son lavados con agua potable o agua clorada a temperatura ambiente en lavadoras industriales de fruta durante alrededor de un minuto. Durante este proceso el higo seco absorbe agua, especialmente la piel, incrementándose el contenido de humedad entre un 2-3% (Şen, 2022).

Tras este proceso, los higos son escaldados para mejorar su apariencia visual y su textura (Figura 22). Este tratamiento es de aproximadamente un minuto y se realiza en balsas de agua a 70-100ºC. En algunas industrias utilizan escaldadoras de vapor, como alternativa, haciendo pasar los higos por una atmósfera de vapor saturado a alta temperatura.

Figura 22. Lavado y escaldado de higo seco. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

A la salida del escaldado, la temperatura de los frutos se reduce rápidamente pasando por duchas de agua a temperatura ambiente. Tras el aumento de humedad en las últimas etapas, es necesario pasar los higos a un túnel de secado donde con la ayuda de ventiladores y aire caliente a una temperatura de 45-50ºC se reduce el contenido de agua. Posteriormente, para reducir y/o eliminar la presencia de higos secos contaminados por aflatoxinas (AFs), se pasan por lámparas de luz ultravioleta (UV) de 365 nm de longitud de onda (Figura 23). Las lámparas UV instaladas en cámaras oscuras permiten visualizar a los trabajadores la presencia de higos secos que emiten fluorescencia amarilla verdosa brillante (BGYF), la cual es producida por el ácido kójico, un indicador de la presencia de AFs, ya que los hongos productores de AFs también generan este ácido.

Figura 23. Detección de higos con AFs a través de luz ultravioleta en industria. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

Cuando se detectan este tipo de higos se retiran de la línea de procesado y se destruyen para evitar la contaminación del resto. La emisión de fluorescencia se detecta principalmente en la parte externa del fruto. Por ello, cuando la contaminación no es muy elevada o si las AFs se encuentran en el interior del higo, este método no es eficaz para detectarlas. El límite máximo establecido por la Comisión Europea (CE) para AFs totales (AFB_1, AFB_2, AFG_1 y AFG_2) en los higos secos es de 10 µg/kg, y para la AFB_1 es de 6 µg/kg (EC, 2012), siendo para la ocratoxina A (OTA) un límite máximo de 8 µg/kg (Reglamento 206 nº 2022/1370) (EC, 2022). Tras estas etapas, los higos secos finalizan su procesado o vuelven a ser almacenados. Dependiendo de la demanda del mercado pueden llegar a estar hasta 12 meses en las cámaras. En algunos casos, debido a reinfestaciones por plagas de almacén, es necesario volver a aplicarles un tratamiento de fumigación durante el almacenamiento.

Figura 24. Higo seco enharinado.

En Extremadura, como último paso, los higos son recubiertos con harina de arroz (Galván et al., 2022a). Esta película de harina no solo captura la humedad que pueda aparecer, sino que también oculta los defectos superficiales, resultando al consumidor un producto más atractivo (Figura 24).

Tras pasar a través de un detector de metales, los higos secos son envasados mediante un equipo con pesaje automático (Figura 25). Existen diferentes formatos en cuanto a cantidad y embalaje, siendo el más común las bolsas de plástico de 400-500 g. Aparte de frutos enteros, los higos secos también pueden ser moldeados manualmente y comercializados de multitud de formas, dependiendo de la zona de producción y el destino. Los frutos pueden ser prensados, cortados en láminas, triturados, bañados en chocolate o incluso dispuestos en ristras (Figura 26; E. Tırkaz et al., 2022).

Figura 25. Envase comercial de higos secos

Figura 26. Diferentes presentaciones comerciales de higo seco. a) chocolate, b) prensado, c) triturados y d) en ristra. Fuente: www.lapasion.es / www.alkarfood.com

6. Agentes bióticos alterantes de los higos secos.

La producción de higos secos de alta calidad es una tarea ardua. Durante su proceso de producción, los frutos son susceptibles de ser alterados por diferentes agentes, tanto bióticos (pájaros, insectos y hongos) como abióticos. Entre los factores ambientales, las temperaturas extremas, la sequía, la lluvia, el granizo y el viento pueden influir de

forma negativa en el tamaño, la calidad y la productividad de los higos secos obtenidos (Flaishman, 2022). Por otro lado, aunque el marchitamiento comience en el árbol y la actividad metabólica continúe, durante el secado al aire libre los frutos son vulnerables a la proliferación de microorganismos, así como al ataque de diversos insectos, aves u otros patógenos (Belessiotis and Delyannis, 2011; Chand et al., 2015). La identificación y el seguimiento de estas plagas y enfermedades, así como el conocimiento de las condiciones ambientales favorables para su crecimiento y reproducción, sus modos de infestación y sus áreas de distribución, son extremadamente importantes para el desarrollo de estrategias eficaces de control (Turanlı, 2022).

6.1 Aves

En la península ibérica, las aves representan la principal amenaza para el cultivo de la higuera. Esta plaga, al alimentarse de los higos, afecta negativamente a la cosecha, haciéndola prácticamente inservible (Figura 27; Casadomet et al., 2016). En la Tabla 2 se indican las principales aves relacionadas con el consumo de higos y daños que ocasionan.

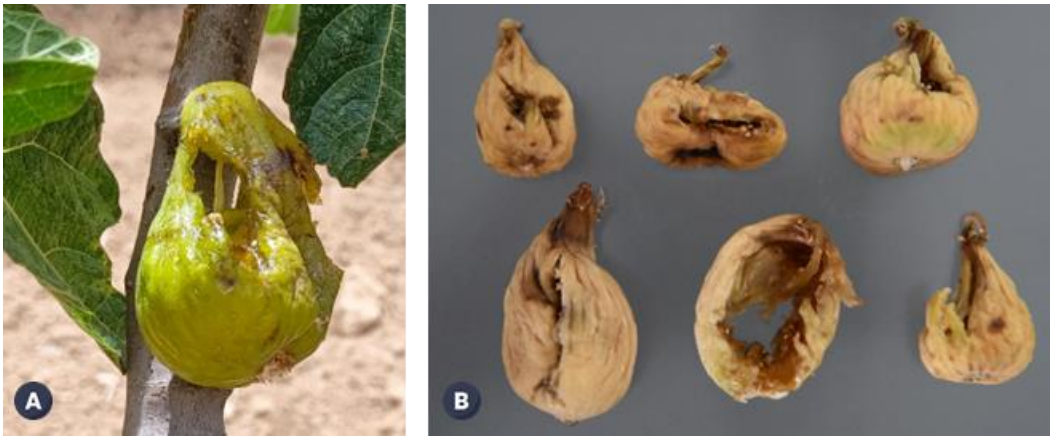


Figura 27. Higos picados por pájaros a) Fresco en el árbol b) Secos. Fuente: Dpto. Fruticultura mediterránea (CICYTEX).

Las aves picotean higos desarrollados, particularmente cuando alcanzan su plena madurez. Por esta razón, en el caso de los higos destinados al consumo en fresco, al recolectarse diariamente y permanecer menos tiempo el fruto maduro en el árbol, se

observan menores mermas que en los higos secos. Los daños en frutos, dependiendo de las especies, van desde pequeñas lesiones por las garras o picotazos, hasta su consumo completo e incluso arrancando el fruto de la higuera. Una vez que las aves localizan esta fuente de alimento en un higueral, es probable que regresen durante toda la campaña, convirtiéndose en un grave problema para el agricultor. Thomas (1979) determinó que la curruca mosquitera (*Silvya borin*) durante su migración otoñal consumía higos de forma abundante a su paso por la zona sur de Portugal, en su preparación para cruzar el Sahara (Figura 28). Es fácil observar a esta especie en grupos de 5-6 individuos alimentándose simultáneamente de pequeñas higueras.

Figura 28. Curruca mosquitera junto a higo fresco picado. Fuente: https://www.malaga.es/medioambiente/

Aun así, las aves desempeñan un papel importante en el ecosistema de la higuera; estos vertebrados actúan como dispersores naturales de semillas de los higos y algunas especies incluso contribuyen al control biológico de plagas al ingerir insectos. Pérez (1983), en su estudio centrado en la ornitología en la vegetación de Extremadura, relacionó a la higuera con el estornino negro (*Sturnus unicolor*), el rabilargo (*Cyanopica cyaneus*) y la urraca (*Pica pica*). Siguiendo la misma línea, Casadomet et al., (2016) identifican al estornino y el rabilargo como las principales aves plaga del higo en España. En la zona sur de américa (Argentina), la avifauna relacionada con la higuera es más amplia, alcanzando 10 especies repartidas en el Orden Passeriformes, Piciformes, Psittaciformes y Tyrannidae (De la Peña and Pensiero, 2017; Berón et al., 2020). Existen varios estudios que relacionan los frutos de diferentes especies del género *Ficus* como importante fuente de alimento para poblaciones de aves (Lambert, 1989; Eshiamwata et

al., 2006; Daru et al., 2013). Generalmente, los higos secos muy dañados por aves se dejan en el higueral durante la recolección o se retiran antes de llevarlos a la industria.

En algunos estudios realizados en higuerales de Extremadura el daño por aves en brevas e higos fresco osciló entre 11 y 30% (Pereira et al., 2015). En cuanto a higo seco, entre el 29,3% y 55,7% del total de los daños registrados eran atribuidos a aves (Galván et al., 2021). Sin embargo, en un estudio realizado en Argentina, los daños fueron inferiores a los descritos anteriormente, con valores máximos de 9,7% de la cosecha de higos frescos (Berón et al., 2020).

Tabla 2. Principales especies de aves relacionadas con la higuera en diferentes áreas de producción y daños que ocasionan en el fruto.

	Especie	Nombre común	Orden: Familia	Referencia	Localización	Daños
Aves	*Sturnus unicolor*	Rabilargo	Passeriformes: Sturnidae	Casadomet et al., (2016) Chiscano, (1983)	España	- Daños en higos próximos a madurez o al alcanzarla. - El daño desde pequeñas marcas de garras o picotazos hasta su consumo completo. Inservible para su comercialización. - Una vez localizan el higueral, se convierten en problema persistente. - Consideradas especies beneficiosas, por la ingestión de insectos, los higos les atraen enormemente.
	Cyanopica cyanus	Tordo o estornino	Passeriformes: Corvidae			
	Pica pica	Urraca	Passeriformes: Corvidae	Chiscano, (1983)		
	Sylvia borin	Curruca mosquitera	Passeriformes: Sylviidae	Thomas, (1979) Lisci and Pacini, (1994)	Portugal	
	Mimus saturninus	Calandria grande	Passeriformes: Mimidae	Berón et al., (2020) Peña and Pensiero, (2017)	Argentina	
	Myiopsitta monachus	Cotorra argentina	Psittaciformes: Psittacidae			
	Paroaria coronata	Cardenal común	Passeriformes: Thraupidae			
	Thraupis sayaca	Celestino	Passeriformes: Thraupinae			
	Melanerpes cactorum	Carpintero cardón	Piciformes: Picidae	Peña and Pensiero, (2017)		
	Passer domesticus	Gorrión	Passeriformes:Passeridae			
	Pitangus sulphuratus	Benteveo común	Tyrannidae: Tyranninae			
	Pipraeidae bonariensis	Naranjero	Passeriformes: Thraupidae			
	Turdus rufiventris	Zorzal colorado	Passeriformes: Turdidae			
	Zonotrichia capensis	Chingolo	Passeriformes: Emberizidae			

6.2 Insectos: dípteros, coleópteros y lepidópteros

Otro de los principales agentes bióticos que ocasiona pérdidas relevantes en la producción de higo seco son los insectos. El cultivo de la higuera es infestado por unas 100 especies de insectos y ácaros (Ben-Yakir and Costa, 2022). Estos pueden infestar el tronco, la corteza, las ramas, las hojas y los frutos, antes y después de ser cosechados, dependiendo del tipo de plaga.

En relación con las plagas de dípteros de la higuera, estas pertenecen principalmente a las familias Tephritidae, Drosophilidae y Lonchaeidae (Tabla 3). La mosca negra del higo, *Silba adipata* Mc Alpine, y la mosca del higo, *Silba virescens* Macquart, son unas de las especies que atacan en los primeros estadíos de desarrollo del higo. Las hembras adultas son atraídas por el latex de los higos sin madurar (Katsoyannos, 1983), ovipositando en las brácteas del ostiolo. Cuando los huevos eclosionan, las larvas son blanquecinas y se alimentan en grupo creando galerías en el interior del siconio. Al completar su desarrollo, las larvas salen a pupar en el suelo, creando orificios visibles en el fruto (Figura 29). Aparte de la disminución de la calidad por los daños interiores y exteriores en el fruto, estos caen del árbol antes de madurar, todavía verdes, convirtiéndose en pérdidas de producción para el agricultor (Ben-Yakir and Costa, 2022). *S. adipata* y *S. virescens* son consideradas monófagas del cultivo de la higuera (Katsoyannos, 1983; Casadomet et al., 2016). La mosca mediterránea de la fruta, *Ceratitis capitata* (Wiedemann), es una plaga polífaga altamente invasiva y destructiva. (Gonçalves et al., 2008). Con una amplia distribución

Figura 29. Daño en fruto de S. virescens a) orificio de salida de larva b) galería en el interior.

geográfica, se puede encontrar en la mayoría de las zonas tropicales y subtropicales del mundo, excepto en Estados Unidos que solo está presente en Hawái (USDA, 2015). Esta mosca es atraída por frutos maduros, en ellos oviposiciona creando una herida al introducir el oviscapto (Figura 30). Las larvas emergentes se alimentan en el interior del fruto creando galerías y descomponiendo la pulpa. Del mismo modo que *Silba* spp, la mosca mediterránea de la fruta al finalizar su estado larvario sale del fruto y pupa en el suelo. Al descomponerse, los higos sobremaduros caen al suelo con larvas en su interior y sin finalizar el secado, se quedan "aguados", no siendo válidos para la recolección. Otra especie similar es la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel), que es la principal plaga del higo en la zona de Asia, países de África subsahariana y algunas islas del pacífico (Singh et al., 2022).

Figura 30. Daños en frutos por *C. capitata.* a) posada en fruto maduro b) oviposicionando en fruto c) larvas en el interior del fruto. Fuente: Dpto. Fruticultura Mediterránea (CICYTEX) / ww.sygenta.es.

La mosca del vinagre, *Drosophila melanogaster* (Meigen), puede causar podredumbre y fermentaciones en los higos maduros y secos. Esta mosca es atraída por los volátiles emitidos por higos y subproductos de la fermentación (Ben-Yakir y Costa, 2022), produciendo, normalmente, daños en frutos almacenados y mal conservados (Casadomet et al., 2016). Las hembras adultas depositan sus huevos en residuos de fruta y en frutos en mal estado, estos frutos pueden transmitir otros organismos que deterioran

la fruta sana haciéndola inservible para su comercialización (Singh et al., 2022). Los adultos de *D. melanogaster* transmiten levaduras a los higos en los que penetran y, cuando las concentraciones de agua y azúcar son correctas, se produce una rápida fermentación o "agriado" (Condit, 1947). Las variedades de maduración tardía son las más susceptibles a estos daños.

En general, la acción de estas moscas no se debe al daño directo causado sino a las fermentaciones que promueven al transmitir microorganismos en la piel del higo.

Tabla 3. Lista de las principales plagas del higo del orden Díptera, daños que causan y área de producción a la que se asocian.

	Especie	Familia	Figura	Nombre común	Daños	Localización	Referencia
Orden: Díptera	*Ceratitis capitata* (Wiedmann)	Tephritidae		Mosca mediterránea de la fruta	- Entrada de microorganismos por orificio de oviposición - Manchas blandas y húmedas en la piel - Desarrollo de larvas en interior - Descomposición de la pulpa del fruto.	España, Portugal, Sudáfrica, Egipto, Argentina, Siria, Túnez, Iraq, Turquía y Albania	Casadomet et al. (2016); Gonçalves (2017); Wohlfarter et al. (2011); Amin and Saafan (2018); Sanchez et al. (2016); Mansour and Mohamad (2016); Howell et al. (1975); Khalaf et al. (2011); Akşit et al. (2003); Gëzhilli et al. (2018)
	Drosophila melanogaster Meigen	Drosophilidae		Mosca del vinagre	- Ataca a frutos sobremaduros, mal almacenados o conservados. - Provoca pudrición y fermentación de los frutos.	México, Turquía, España, Italia, Grecia y California (EE. UU.)	Bautista et al. (2017); Başpinar et al. (2022); Casadomet et al. (2016); Baser et al. (2014); Triantaphyllidis and Tsacas (1981); Miller and Phaff (1962).
	Silba adipata McAlpine	Lonchaeidae		Mosca negra del higo	- Oviposición en el ostiolo de higos sin madurar, entrada de microorganismos - Las larvas realizan galerías en el interior del fruto - Caída de frutos sin madurar	España, Sudáfrica, Israel, Grecia, California (EE. UU.), Japón, Túnez, Turquía y Portugal	Costa (2019); Giliomee et al. (2007); Nawade et al. (2020); Katsoyannos (1984); Britt et al. (2022); Arimoto et al. (2020); Abbes et al. (2021); Talhouk (2003); Gonçalves (2017)
	Silba virescens Macquart	Lonchaeidae		Mosca de los higos		Egipto, España e Israel	Saafan et al. (2000); Casadomet et al., (2016); MacGowan and Freidberg (2008)

Otro de los grupos de insectos que causan daños relevantes en el higo son los coleópteros (Tabla 4). Entre las principales plagas de este grupo de insectos del higo seco, se encuentra *Carpophilus hemipteurs* (L.) (Figura 31), conocido como escarabajo de los frutos secos o escarabajo de la sabia. Esta plaga se extiende por todo el mundo, causando daños tanto en higos maduros en el árbol como en los secos en el suelo o almacén. Los adultos de *C.hemipterus* tienen un tamaño pequeño (~3 mm) y se caracterizan por tener manchas amarillentas en sus élitros (Casadomet et al., 2016). Los escarabajos adultos penetran en los higos maduros o en proceso de secado, pudiendo acceder por el ostiolo sin causar daños externos visuales. Los escarabajos adultos mediante la inoculación de levaduras en el fruto causan la fermentación de los tejidos creando condiciones favorables para su alimentación y posteriormente de las larvas. La hembra tras aparearse deposita en el interior del fruto los huevos (Ferguson et al., 1990). Estos escarabajos pueden dañar los higos reduciendo su calidad, favoreciendo la contaminación por microorganismos y haciendo más atractiva a la fruta para otras plagas (Ben-Yakir y Costa, 2022). Todas las variedades son susceptibles a ser dañadas por esta plaga, sin embargo, las que tienen un ostiolo más pequeño suelen verse menos afectadas (Coviello and Bentley, 2009). De la familia Nitidulidae, *C. hemipterus* es la especie más conocida como plaga de la higuera, pero *Carpophilus freemani* y *Carpophilus mutilatus* también son comunes.

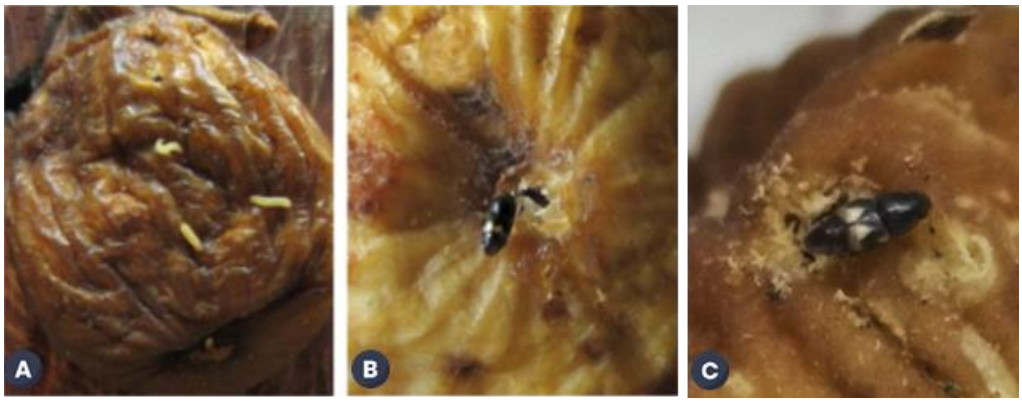


Figura 31. Presencia *C. hemipterus*. a) larvas b) adulto entrando por el ostiolo c) adulto Fuente: Casadomet et al., 2016 / Dpto. Fruticultura mediterránea (CICYTEX).

Otro coleóptero carpófago que también se alimenta del fruto es *Gonocephalum pusillum* Fabricius o falso gusano del alambre. El adulto mide entre 8-9 mm de longitud, de color negruzco y con los élitros paralelos y con estrías delimitadas por series de puntos grandes y profundos. Este escarabajo causa daños directos al higo cuando se encuentra en el suelo finalizando el secado antes de la recolección (Figura 32). Los individuos adultos se encuentran bajo tierra a pocos centímetros de la superficie, a la que ascienden para alimentarse. Las mordeduras mayoritariamente son superficiales, aunque en algunas ocasiones son más profundas provocadas por el insecto adulto. Los frutos atacados por esta especie no son comercializados en formato de fruto entero, siendo necesario destinarlos a producto elaborado de pasta o trozos de higo (Gragera-Facundo, 2014; Casadomet et al., 2016). Gragera-Facundo (2014) en un estudio en un higueral de secano, situado en el centro- de Extremadura, determinó que el 60% de la plantación había sido infestada por este escarabajo.

Figura 32. Higos dañados por G. pusillum. Fuente: Gragera-Facundo, 2014.

Otras especies de coleópteros que desarrollan su actividad mayoritariamente en los almacenes y que causan daños en el higo seco son los gorgojos: *Tribolium castaneum* (Herbst), *Tribolium confusum* Jacquelin du Val, *Tenebrio molitor* L. y *Sitophilus granarius* (L.). Los frutos dañados aparecen con agujeros, erosiones y con excrementos, quedando inservibles para la venta (Casadomet et al., 2016).

Tabla 4. Lista de las principales plagas del higo del orden Coleoptera, daños que causan y área de producción a la que se asocian.

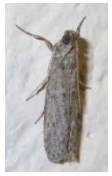
	Especie	Familia	Figura	Nombre común	Daños	Localización	Referencia
Orden: Coleoptera	*Carpophilus hemipterus* (L)	Nitidulidae		Escarabajo de la fruta seca	- Se alimenta en el árbol, en el suelo o en el almacén de higos tanto frescos como secos. - Vector de entrada de microorganismos. - Restos de individuos muertos y excrementos en el interior del fruto.	España, California (EE. UU.), Grecia, Turquía,	Casadomet et al. (2016); Burks and Brandls (2005); Eliopoulos and Athanassiou (2004); Turanlı (2003)
	Gonocephalum pusillum Fabricius	Tenebrionidae		Falso gusano del alambre	- Se alimenta de los higos secos en el suelo del higueral. - Daño superficial importante, frutos no comercializables.	España	Gragera-Facundo (2014); Casadomet et al. (2016)
	Tribolium castaneum (Herbst)	Tenebrionidae		Escarabajo rojo de la harina	- Se alimenta del higo seco durante el almacenamiento. - Frutos con orificios y excrementos en su interior,	España y California (EEUU)	Casadomet et al. (2016); Johnson et al. (2000)
	Tenebrio molitor L.	Tenebrionidae		Gusano de la harina		España	Casadomet et al. (2016)
	Tribolium confusum Jacquelin du Val	Tenebrionidae		Falso gorgojo de la harina			

Por último, en relación con los insectos también hay que destacar las plagas del orden Lepidóptera, principalmente son polillas de la familia Pyralidae las que atacan al fruto durante el secado y almacenamiento (Tabla 5). Entre ellas una de las más extendidas en el sector del higo seco es la polilla del higo, *Ephestia cautella* (Walker). La hembra adulta de esta polilla tras aparearse deposita sus huevos entre los frutos. Durante todo el desarrollo larvario, esta especie se alimenta de higos secos y cuando las larvas maduran salen del fruto para pupar en un capullo de seda sobre la superficie de las paredes, esquinas de cajas o entre los frutos (Turanlı, 2022). Además de la polilla mencionada, otras especies ampliamente extendidas son: la polilla de las pasas, *Ephestia figulillela* (Gregson), la polilla de la harina, *Ephestia kuehniella* Zeller, la polilla del algarrobo, *Ectomyelois ceratoniae* Zeller, y la polilla india de la harina, *Plodia interpunctella* (Hübner). El gusano de la naranja Navel, *Amyelois transitella* (Walker), exclusivamente localizado en América es una de las principales plagas de los higos secos californianos. En un estudio realizado por Burks and Brandl (2005) estimaron que esta especie era responsable del 32% de los daños registrados por insectos en los frutos.

Todas estas polillas causan daños en los frutos tanto al alimentarse como al salir del fruto creando orificios (Figura 33). Sin embargo, el daño principal se debe a la presencia de sedas, heces, cutículas, restos de insectos o huevos en los frutos, además de la pérdida de producto y la posible contaminación por hongos saprófitos.

Figura 33**.** a) larva de *E. figulilella* en el interior del higo seco b) orificio de salida de larva.

Tabla 5. Lista de las principales plagas del higo del orden Lepidóptera, daños que causan y área de producción a la que se asocian.

	Especie	Familia	Figura	Nombre común	Daños	Localización	Referencia
Orden: Lepidóptera	*Ephestia cautella* (Walker)	Pyralidae		Polilla de la almendra o higo	- Los higos, principalmente secos, son parasitados tanto en el campo como durante el almacenamiento. - Las larvas se alimentan en el interior del fruto, contaminándolo con heces y sedas. - Las larvas realizan orificios de entrada y salida, aunque esto puede realizarse por el ostiolo. - Pupan tanto en el interior como en el exterior de los frutos.	Turquía, Grecia y California (EE. UU.)	Turanlı, 2003; Eliopoulos and Athanassiou, 2004; Johnson et al., 2020;
	Ephestia figulillela (Gregson)	Pyralidae		Polilla de las pasas		Grecia, Turquía, Irán y California (EE. UU.),	Eliopoulos and Athanassiou, 2004; Emekçi and Ferizli., 2000 Jalili et al., 2004; Johnson et al., 2020;
	Ephestia kuehniella Zeller	Pyralidae		Polilla de la harina		España, Irán, y California (EE. UU.),	Casadomet et al., 2016; Sadeghi et al., 2018; Johnson et al., 2020;
	Ectomyelois ceratoniae Zeller	Pyralidae		Polilla del algarrobo		España e Irán	Costa, 2019; Norouzi et al., 2008;
	Amyelois transitella (Walker)	Pyralidae		Gusano de la naranja navel		California (EE. UU.), Argentina y Brasil	Burks and Brandl, 2005; Singh et al., 2022
	Plodia interpunctella (Hübner)	Pyralidae		Polilla india de la harina		España, Grecia, Turquía, Irán y California (EEUU),	Casadomet et al., 2016; Eliopoulos and Athanassiou, 2004; Cetinkaya et al., 2006; Jalili et al., 2004; Johnson et al., 2020;

6.3 Hongos filamentosos: mohos

La contaminación y desarrollo fúngico es una problemática extendida entre una amplia gama de alimentos de baja-intermedia humedad, ente ellos se encuentran los frutos secos, cereales y especias. Los mohos son capaces de crecer dentro de una amplia gama de $a_{w,}$ valores de pH y temperaturas, utilizando de sustrato los carbohidratos y siendo más competitivos que otros microorganismos en esas condiciones. (Huis in't Veld, 1996; Medina et al., 2015ab). Los hongos xerófilos se caracterizan por ser capaces de crecer rápidamente por encima de 0,77 y de crecimiento lento de 0,75 hasta aproximadamente 0,68 de $a_{w,}$ (Pitt and Hocking, 2022).

La producción de higos secos implica algunas prácticas agrícolas específicas que presentan riesgos significativos de infección fúngica de los frutos y que puede acabar en contaminación por micotoxinas (Gilbert and Senyuva, 2008). Como se ha explicado anteriormente, los higos maduran en el árbol, comienzan a marchitarse y caen al suelo para finalizar su secado al sol, donde permanecen entre 7 y 10 días. Durante estas fases, donde se dan condiciones óptimas para el crecimiento de hongos de a_w (~ 0,8) y temperatura (25-30ºC), la infestación fúngica puede producirse fácilmente, ya sea a través del polvo, transmisión por insectos y aves o directamente del suelo (Gilbert and Senyuva, 2008). Del mismo modo, las condiciones de temperatura y a_w varían durante el procesado industrial del higo seco, pudiendo presentar igualmente riesgos de infestación fúngica (Galván et al., 2022a). Esta susceptibilidad que tiene el higo seco a la contaminación por mohos ha llevado a que se realicen diferentes estudios entorno a esta problemática en diferentes zonas de producción del mundo. En la Tabla 6 se muestran los principales géneros y especies de mohos asociados a la mycobiota del higo seco de diferentes áreas de producción, así como los niveles encontrados.

Tabla 6. Niveles de mohos, principales especies identificadas y micotoxinas detectadas en higos secos de diferentes países.

	Descripción	Especies	Recuentos / Micotoxinas	Referencia
España	Z: Extremadura (4 higuerales) T: Recolectados campo V: Calabacita	**Abundancia de mohos aislados:** 29,4% *Penicillium* spp. 24,5% *Aspergillus* spp. 18.9% *Cladosporium* spp. 17,3% *Alternaria* spp.	**Recuentos:** 2,43 – 2,85 log ufc/g **Micotoxinas:** -AFB1+AFB2: 10.7% muestras -OTA: 12,5% muestras	Galván et al. (2023)
	Z: Extremadura (3 Industrias) T: Proceso industrial V: Calabacita	**Abundancia de mohos aislados:** 27,7% *Aspergillus welwitschiae* 16,5% *Aspergillus tubingensis* 16,5% *Aspergillus flavus* 11,1% *Penicillium citrinum* 5,6% *Aspergillus niger* 5,6% *Aspergillus transmontanensis* 5.6% *Penicillium expansum* 5,6% *Penicillium bilaiae* 5,6% *Penicillium crustosum*	**Recuentos:** 2,03 – 3,10 log ufc/g **Micotoxinas:** -AFB1+AFB2: 12,5% muestras de una de las 3 industrias -OTA= 0% muestras	Galván et al. (2022a)
Irán	Z: Estahban región T: Recolectados campo y proceso industrial	**Incidencia en muestras:** 90,9% *Aspergillus niger* agg 63,7% *Aspergillus flavus* *54,6% Acremonium* spp. 36,7% *Aspergillus* spp. 28,3%*Mucor* spp. *9,1% Penicillium* spp. / *Alternaria* spp.		Javanmard (2010)
India	Z: Amravati T: Producto final	**Incidencia en muestras:** 52,7% *Eurotium chevalieri* 27,2% *Aspergillus versicolor* 13,4% *Penicillium nigricans* 8,8% *Cladosporium cladosporioides* 5,4% *Fusarium solani* 5,3% *Penicillium oxalicum* 2,5% *Aspergillus niger* 2,2% *Aspergillus candidus* 2,0% *Aspergillus parasiticus*		Hedawoo et al. (2017)
Iraq	Z: Kurdistán T: Producto final	**Incidencia en muestras:** 73,4% *Apergillus niger* 66,0% *Aspergillus flavus* 31,3% *Aspergillus carbonarius* 31,3% *Aspergillus parasiticus* 11,3% *Penicillium glabrum* 7,0% *Penicillium expansum* 6,0% *Penicillium verrucosum*		Saadullah and Abdullah (2015)
Yemen	Z: Gobernación de Ibb y Adan T: Producto final	**Abundancia de mohos aislados:** 28,1% *Aspergillus niger* 18,8% *Aspergillus flavus* 12,5% *Rhizopus stolonifer* 9,4% *Aspergillus fumigatus* 9,4% *Penicilium chrysogenum* 6,25% *Eurotium amstelodami*	**Micotoxinas:** En 20 muestras -OTA: 2 positivas (70-160 µg/kg) -AFB1: 2 positivas (120-250 µg/kg)	Alghalibi and Shater (2004)

		3,3% *Cladosporium cladosporoides* 12,5% Otros		
Turquía	Z: Aegean region T: Recolectados campo	**Incidencia en 115 muestras:** 76,5% *Fusarium* spp. 65,2% *Aspergillus niger* agregado 40,0% *Aspergillus flavus* 39,1% *Aspergillus foetidus* 21,7% *Aspergillus carbonarius* 8,7% *Aspergillus awamori* 5,2% *Aspergillus terreus* 4,3% *Penicillium chrysogenum* 4,3% *Aspergillus parasiticus*	**Recuentos:** < 2,0- 7,5 log ufc/g **Micotoxinas:** En 115 muestras: - OTA: 48% positivas (0.1–15.3 ng/g) -FB1: 74,7% positivas (0.05–3.65 mg/kg) -AFS: 10% positivas (0.1– 763.2 ng/g) -CPA: 24,3% positivas (25–187 ng/g)	Heperkan et al. (2012)
	Z: Izmir T: higos con fluorescencia bajo luz U.V en la industria V: Sarilop	**Incidencia en 50 muestras:** 44 *Aspergillus niger* 21 *Aspergillus flavus* 20 *Penicillium expansum* 18 *Eurotium amstelodami* 13 *Aspergillus Ochraceus* 11 *Fusarium culmorum* 9 *Fusarium* spp.	**Micotoxinas:** En 50 muestras -OTA: 32 positivas (0,7 - 1710 ng/g) -AFB1: 49 positivas (0,6-2221 ng/g). -AFB2: 30 positivas (0,1-74,4 ng/g) -AFG1: 29 positivas (0,2-734,2 ng/g) - AFG2: 4 positivas (0,2-22,6 ng/g)	Şenyuva et al. (2008)
California (EEUU)	Z: Fresno, Condado de Madera y Merced T: Higos recolectados campo (4 años de estudio) V: Calimyrna y Conadria	**Incidiencia de hongos esporulados:** 5,05% *Aspergillus* section *Nigri* 0,10% *Eurotium amstelodami* 0,06% *Aspergillus terreus* 0,06% *Eurotium chevalieri* 0,04% *Aspergillus flavus* 0,04% *Aspergillus ochraceus* 0,04% *Aspergillus tamarii*	**Micotoxinas** En 15 frutos infectados de *Aspergillus* Section *Circumdati* -OTA: 40% positivo (19-9600 ng /g) En 31 higos secos infectados de *Aspergillus* Sección *Flavi* -AFs: 38,7% positivo	Doster et al. (1996)

En cuanto a estudios de higos secos directos del campo, Heperkan et al., (2012) reportaron que un 94,7% de muestras de higos turcos contenían especies de mohos micotoxigénicos, siendo las especies más frecuentes detectadas las pertenecientes a *Aspergillus* sección *Nigri* (93%), seguidos de *Fusarium* spp., *Aspergillus* sección *Flavi* y *Penicillium* spp. En la zona de California, se identificaron en higos secos con hongos

visibles 23 especies de *Aspergillus* spp., siendo las principales causantes de las esporas negras de la "enfermedad del tizón", *A. niger, A. awamori* (actualmente *Aspergillus welwitschiae*)*, A japonicus* y *A. carbonarius* (Doster et al., 1996). En España, Galván et al., (2023) tras determinar la población fúngica de diferentes zonas productoras de Extremadura, de higuerales en secano y regadío, reportó de la presencia de 40 especies de mohos, pertenecientes a *Penicillium* spp. (29,4%), *Aspergillus* spp. (24,5%), *Cladosporium* spp. (18,9%) y *Alternaria* spp. (17,3%). También, en un estudio realizado durante el procesado de higo seco en industrias de las mismas zonas, encontraron que el género *Aspergillus* spp. predominó en la mayoría de las etapas, excepto durante el escaldado donde prevaleció *Penicillium* spp. (Galván et al., 2022). En un estudio similar en una industria iraní, Javanmard (2010) informó que las especies predominantes eran *A. niger, A. flavus, Acremonium* spp. y *Mucor* spp. en porcentajes de 90.9%, 63.7%, 54.6% y 36.4%, respectivamente. En cuanto a producto final, estudios realizados en higos adquiridos en mercados de Iraq y Yemen encontraron que *A. niger* y *A. flavus* eran las principales especies detectadas (Alghalibi and Shater, 2004 ;Saadullah and Abdullah, 2015) Sin embargo, Hedawoo et al., (2017) en higos comprados en India determinaron como principal especie a *Eurotium chevalieri*, 52,7%, seguida de *Aspergillus versicolor, Penicillium nigricans*, *Cladosporium cladosporioides* con porcentajes del 27,2%, 13,4% y 8,8%, respectivamente. Por tanto, en base a los diversos estudios realizados en diferentes zonas de producción de higo seco en muestras tomadas en las distintas fases del proceso productivo se puede concluir que las especies del género *Aspergillus* de la sección *Flavi* y sección *Nigri* son las predominantes en este producto.

La mayoría de las especies fúngicas nombradas en párrafos anteriores se caracterizan por la producción natural de micotoxinas, siendo el principal objeto de preocupación para los productores e industriales.

Las micotoxinas son metabolitos secundarios de bajo peso molecular, producidos por ciertos géneros de mohos filamentosos y pueden encontrarse en alimentos causando riesgos para animales y seres humanos (Özer, 2022). Hasta ahora se han aislado e identificados químicamente más de 400 micotoxinas (Asghar et al., 2017; Ünüsan, 2019). Sin embargo, los investigadores se han centrado principalmente en las que causan un daño significativo para los seres vivos, como aflatoxinas (AFs), ocratoxinas (OTA y OTB), patulina, las fumonisinas, zearalenona, nivalenol y desoxinivalenol. Los efectos de las micotoxinas en los organismos vivos son muy diversos y pueden tener importantes efectos sobre la salud, como ser carcinógenas, mutagénicas, dañinas para el ADN, obstaculizar la síntesis de ARN y proteínas, causar malformaciones y lesiones cutáneas y suprimir el sistema inmunitario (Özer, 2022).

En la bibliografía analizada son varios los estudios sobre su presencia en higos secos de distintos orígenes y diferentes calidades, desde sanos hasta con fluorescencia (BGYG) e incluso frutos podridos y mohosos. Estos trabajos han revelado que los higos secos pueden contener una amplia variedad de micotoxinas consecuencia de la gran diversidad de mohos identificados en este producto (Steiner et al., 1988; Doster et al., 1996; Karaca and Nas, 2006; Heperkan et al., 2012; Di Sanzo et al., 2018; Sulyok et al., 2020; Galván et al., 2023). Entre ellas, se encuentran AFs, OTA, fumonisinas (FBs), ácido kójico (KA), zearalenona (ZEA), toxina HT-2 (T2), el ácido tenuazónico (TeA), el alternariol (AOH), el ácido ciclopiazónico (CPA), patulina (PAT), ácido fusárico (FA), beauvericina (BEA), fusaproliferina (FP) y ergosterol (ERG) (Karaca and Nas, 2008; Gilbert and Şenyuva, 2008; Di Sanzo et al., 2018; Galván et al., 2021). *Aspergillus, Penicillium, Alternaria* y *Fusarium* son las principales especies responsables de la formación de micotoxinas (Bhatnagar et al., 2004; Galván, 2022). En higos secos recolectados en Extremadura el 10,8% estaban contaminados con AFs y un 12,5% de

OTA, ambas en un rango de 0,1 a >70 ppb (Galván et al 2023). Bircan (2009) determinó que el 18% de higos muestreados de origen turco tenían presencia de OTA en una cantidad de 0.51–58.04 ng $^{g-1}$. Di Sanzo (2018) detectó en 41 de las 55 muestras analizadas de higos secos adquiridos en comercios italianos, al menos, una de las micotoxinas investigadas (AFs, OTA, Fumonisina B_1, FA y BEA). Más recientemente, Sulyok et al. (2020) identificaron y cuantificaron 43 metabolitos fúngicos en 180 higos secos de Turquía, incluidas ocho micotoxinas reguladas por la UE que emitían fluorescencia bajo luz UV. Estos estudios, están en correlación con los mostrados anteriormente sobre las principales especies de mohos identificadas en higo seco, donde las especies de mohos del género *Aspergillus* productoras de AFs y OTA estaban entre las predominantes, lo cual supone un grave problema de seguridad alimentaria.

Con el objetivo de proteger a los consumidores frente a riesgos de seguridad alimentaria, varios países como EEUU, Australia y Japón tienen sistemas de información pública que informan de las irregularidades detectadas en los controles de mercado o en la fase de importación. En el caso de la Unión Europea (UE) se dispone del Sistema de Alerta Rápida para Alimentos y Piensos (RASFF) para la rápida comunicación de los peligros detectados en alimentos entre países de la UE. Este sistema durante el año 2022 notificó un total de 69 alertas en higos secos, de las cuales 46 fueron por superar los niveles de AFs y 23 por los niveles de OTA. Del total de alertas, solo una de OTA fue de higos secos de origen español y las restantes en higos secos procedentes de Turquía.

7. Métodos de control de plagas y enfermedades del higo seco.

La prevención y el control de plagas y enfermedades en la producción de higos secos es un aspecto crucial para garantizar un producto de calidad y seguro para el consumidor, al mismo tiempo para reducir las pérdidas por los daños ocasionados por las

mismas. Su gestión integrada es el principal enfoque para controlar las plagas y enfermedades de los higos y minimizar el impacto medioambiental. Este enfoque incluye una combinación de métodos como los agentes biológicos, los productos químicos blandos, la agricultura de precisión, el momento óptimo de aplicación de productos químicos y las prácticas culturales (Mendoza- Castillo et al., 2017).

En la Tabla 7 se muestran diferentes técnicas para la prevención y control de plagas y enfermedades durante el desarrollo del fruto, secado y recolección del higo seco. Las condiciones del cultivo de los frutos son importantes para la calidad de productos desecados. Bulbul et al. (1998) revelaron en un estudio desarrollado en la principal zona de producción de higos secos de Turquía, que las prácticas culturales juegan un papel muy importante para producir frutos de alta calidad y reducir la proporción de higos desechados.

Tabla 7. Métodos de prevención y control de plagas y enfermedades durante la etapa precosecha.

Medidas de control		Descripción	Agente biótico afectado	Referencia
Físicos	Prácticas culturales	- Poda - Evitar humedad debajo del árbol - Reducir polvo ambiental - Preparar suelo antes de la caída de higos (rastrillado y deshierbe) - Eliminar los frutos o restos de recolecciones pasadas - Cosecha frecuente de frutos	**Mohos** **Insectos**	Mostowfizadeh-Ghalamfarsa et al. (2022); Michailides (2003); Flaishman et al. (2022); Ben-Yakir and Costa, (2022); Singh et al. (2022); Coviello and Bentley, (2009); Codex Alimentarius, (2008)
	Barreras físicas	- Frutos protegidos con redes o cajas - Uso de redes o mosquiteras en árboles	**Insectos** - Moscas - Escarabajos **Aves**	Ben-Yakir and Costa, (2022); Singh et al., (2022); Brien and Hardy, (2002)
	Trampas (monitoreo o capturas masivas)	- Feromonas (sexuales o agregación) - Atrayentes (sulfato de amonio, hidrolizado de proteínas, hexanol, nano-emulsión, compuestos volátiles)	**Insectos** - Polillas - Escarabajos - Moscas	Krittika et al. (2019) Katsoyannos and Guerin (1984); Raz (1998); Ben-Yakir and Costa, (2022); Nawade et al., (2020)

		- Trampa deltametrina/ lambda cihalotrin - Adhesivas amarillas		
	Repelentes visuales o sonoros	- Cintas reflectantes, réplicas de depredadores, cometas y espantapájaros - Reproducción de sonidos de pájaros, cañones y caza	**Aves**	Nievas et al. (2021); Brien and Hardy (2002); Whitam et al., (2007); Krezdorn and Adriance (1961)
	Secado alternativo	- Al aire libre sin contacto con el suelo (Mallas suspendidas o bandejas) - Secadero solar - Artificial (hornos, túneles de secado, cabinas o microondas) - Pretratamientos	**Insectos** - Escarabajos - Polillas **Mohos**	Villalobos et al. (2016); Mat Desa et al. (2019); Galván et al. (2021); Okşar et al. (2017); Piga et al. (2004); Petrić et al. (2018)
Biológicos	Selección de variedades	- Frutos con pequeños ostiolos y densas pulpas	**Insectos** - Escarabajos - Moscas **Hongos**	Coviello and Bentley (2009); Michailides 2003; Doster et al. (2002)
	Liberación de individuos	- Enemigos naturales - Machos estériles	**Insectos** - Polillas - Escarabajos - Moscas	Flores et al. (2013); Kraaijeveld and Chapman (2004); Blumberg (2019); Katsoyannos (1983)
	Aplicación de microorganismos	- Uso de bacterias y levaduras como antagonista. - *A. flavus* atoxigénico - *Bacillus amyloliquefaciens* - *Aureobasidium pullulans* - *Pythium oligandrum* - *Trichoderma asperellum*	**Mohos**	Doster et al. (2014); USEPA (2017); Ruiz-Moyano et al. (2016); MAPA (2023); Öztopuz et al. (2018).
		- *Bacillus thuringiensis* - *Beauveria bassiana*	**Insectos**	Cutino et al. (2023); MAPA (2023); Flores et al. (2013)
Químicos	Químicos autorizados en España	- Aceite de parafina - Aceite de naranja	**Insectos** **Mohos**	MAPA, (2023); Smilanick et al. (1978);
		- Azufre - Hidróxido cúprico - Oxicloruro de cobre	**Mohos**	MAPA (2023)
	Químicos naturales	- Extractos de plantas	**Insectos** Moscas	Ismail et al. (2016);

Técnicas como la poda favorecen la circulación del aire dentro de la copa, lo que ayuda a controlar la humedad relativa a la que se expone el higo (Mostowfizadeh-

Ghalamfarsa, 2022; Palmateer et al., 1999), evitando así condiciones húmedas en las que hongos micotoxigénicos como la *Alternaria* spp. se propagan más fácilmente (Lee et al., 2015b). Del mismo modo, un buen manejo del riego sin zonas encharcadas y reduciendo la dosis de riego días antes del inicio de maduración de los higos evita un ambiente húmedo bajo la copa (Stover et al., 2007). También, que se prolongue el secado de los frutos y se favorezcan condiciones ambientales óptimas para la proliferación de microorganismos (Aksoy, 1981; Can, 2022). Durante el crecimiento de los frutos, deben regarse periódicamente los caminos de tierra cercanos a las higueras, de esta forma se minimizará la contaminación a través del aire, el cual contiene polvo mezclado con esporas o ácaros (Codex Alimentarius, 2008; Mostowfizadeh-Ghalamfarsa, 2022). En este contexto, Michailides et al. (2003) demostraron que escarabajos infestados de *A. niger* y encerrados con higos sanos no aumentaron los niveles de higos con mohos visibles en relación con la fruta inoculada sólo con *A. niger*. Por el contrario, cuando los higos fueron espolvoreados con polvo de tierra y *A. niger*, se produjo un aumento significativo. Otra estrategia agronómica común que ayuda al control de plagas y enfermedades es preparar la superficie del suelo en la parte baja del árbol previamente a la caída de los frutos mediante deshierbe e incluso a veces puede cubrirse con tela o lona de plástico (Özen et al., 2008). Una estrategia más novedosa y eficaz, es la de disponer una malla suspendida en el aire bajo la higuera que evita el contacto de los frutos con el suelo, lo cual consigue una mejora en la calidad higiénico-sanitaria y reduce el daño por insectos en los frutos (Galván et al., 2021).

Una vez que los higos caen, se recomienda que se recolecten frecuentemente evitando así largas exposiciones (Codex Alimentarius, 2008). Los higos secados al aire libre son más propensos a la infestación por insectos, a la alteración por plagas, la contaminación con polvo y al crecimiento microbiano (Mat Desa et al., 2019). Por todo

ello, existen diferentes alternativas de secado, como los secaderos solares cubiertos, que evitan la exposición de los frutos a estos agentes, o el secado artificial, que además permite el control de las condiciones de temperatura y humedad (Mat Desa et al., 2019). Toda la fruta desechada o no recolectada en el higueral debe ser eliminada de los huertos tras la recolección, ya que ayuda a reducir la población de insectos hibernantes y de ser un reservorio de microorganismos (Codex Alimentarius, 2008).

En relación con el control específico de las aves, la efectividad de las diferentes técnicas repelentes propuestas puede variar según las regiones, las especies y los cultivos frutales (Simon, 2008). En el caso de repelentes visuales y sonoros, las aves a los pocos días se acostumbran su presencia o frecuencia perdiendo su efectividad. Sin embargo, siendo utilizados en combinación con otros métodos muestran mejores resultados (Bishop et al., 2003). El método más eficaz y extendido en el cultivo de la higuera es el uso de barreras físicas como el uso de redes (Singh et al., 2022). Sin embargo, en las zonas productoras de higo seco de Extremadura no está en uso por sus altos costes y dificultades a la hora de instalarlo. En esta región el método más efectivo es el control con armas de fuego combinado con el uso de cañones de aire en los higuerales durante la época de cosecha. Ambas técnicas están cada vez más restringidas, siendo necesario permisos de las autoridades competentes para su utilización.

En el control específico de insectos, el monitoreo de estos es importante para aplicar las medidas correctivas precisas en campo. En este sentido debe desarrollarse un plan de seguimiento para la inspección visual o el uso de trampas, comenzando antes de que las plagas comiencen a causar daños en el campo o en la industria (Turanlı, 2022). Existen diferentes tipos de trampas y de atrayentes que se utilizan en base a la especie tanto para el monitoreo como para el control de la plaga, con capturas masivas. Respecto a las feromonas, las sexuales son las más conocidas y utilizadas en el control de plagas.

Suelen ser producidas y emitidas por la hembra, al percibirlas el macho se origina comportamiento de búsqueda (Planello et al., 2015). Otro tipo son las feromonas de agregación, Bartelt (1997) identificó las feromonas producidas por los machos de C. *hemipterus* a las que responden tanto machos como hembras, relacionadas con los volátiles producidos por alimentos en fermentación. Trampas con compuestos químicos como hexanol, sulfato de amonio o proteína hidrolizada son utilizadas para atraer especies de moscas como *S. adipata* (Katsoyannos and Guerin, 1984). Existen también trampas específicas para especies con insecticidas químicos autorizados para la higuera. Así, para el control *C. Capitata* el producto Ceratipack, cuenta con un atrayente y están recubiertas de deltametrin como insecticida (MAPA, 2023).

Las políticas de la Unión Europea (UE) sobre productos químicos y plaguicidas son cada vez más restrictivas, ya que están orientadas a proteger la salud humana y el medio ambiente. Dentro de estas políticas destaca El Pacto Verde Europeo, el cual engloba un paquete de iniciativas cuyo objetivo es situar a la UE en el camino hacia una transición ecológica, con el objetivo último de alcanzar la neutralidad climática de aquí a 2050. A corto plazo dentro de la estrategia "De la granja a la mesa", donde uno de los objetivos principales es "Garantizar la seguridad alimentaria, la nutrición y la salud pública, velando porque todas las personas tengan acceso a alimentos seguros, nutritivos, sostenibles y en cantidad suficiente", en el año 2030 se pretende reducir en un 50% el uso y el riesgo global de los plaguicidas químicos, así como el uso de los plaguicidas más peligrosos (https://www.consilium.europa.eu/es/policies/green-deal/). En España, en el caso de la higuera, en la actualidad hay pocos productos autorizados para el control de plagas o enfermedades, entre ellos se encuentran: aceite de parafina y naranja, azufre, hidróxido cúprico, oxicloruro de cobre, fosfuro de aluminio y fosfuro de magnesio (MAPA, 2023).

En este contexto, en los últimos años en el control de plagas y enfermedades ha despertado un especial interés los métodos biológicos por ser más saludables y respetuosos del medio ambiente. Entre ellos podemos destacar la selección de variedades que produzcan frutos con ostiolos pequeños y densas pulpas que dificultan el acceso de insectos y hongos al interior del fruto (Ben-Yakir and Costa, 2022). La variedad Sarilop, debido a la gran apertura de su ostiolo, es más susceptible a la podredumbre y a los problemas del escarabajo de la fruta seca (Codex Alimentarius, 2008; Coviello and Bentley, 2009). De forma similar, la liberación de individuos, ya sean machos esterilizados o enemigos naturales, ejerce control sobre las plagas evitando la infestación de los frutos. Los machos esterilizados son a menudo utilizados para la supresión de poblaciones de *C. capitata* (Kraaijeveld and Chapman, 2004). En el caso de plagas de lepidópteros, diversos enemigos naturales han demostrado ser efectivos, entre ellos, *Bracon hebetor* Say de la familia Braconidae, *Trichogramma* spp de la familia Trichogrammatidae y *Venturia canescens* Grav., de la familia Ichneumonidae (Hymenoptera) (F. Turanlı, 2022). Algunos de estas especies, como *B. hebector,* han sido detectados en industrias de procesado de higo seco en Grecia (Eliopoulos and Athanassiou, 2004). También la aplicación de microorganismos antagonistas ha mostrado su eficacia en el control de mohos e insectos. La aplicación de *A. flavus* atoxigénicos, como la cepa AF36, ha sido registrada por el departamento de Agricultura de los Estados Unidos como producto de biocontrol para reducir la contaminación de aflatoxinas en diferentes cultivos, entre los que se encuentra la higuera (Doster et al., 2014; USEPA, 2017). Ruiz-Moyano et al. (2016) examinaron frente a *Penicillium expasum* y *Cladosporium cladosporoides* una selección de levaduras antagonistas aisladas de cultivos de brevas e higos, observando que algunas de ellas tenían capacidad para inhibir el crecimiento de los mohos. Entre ellas, determinadas levaduras de las especies

Hanseniaspora uvarum y *Hanseniaspora opuntiae* mostraron capacidad capacidad antifiúngica mediante la producción de compuestos volátiles orgánicos (VOCs: ácido octanoico (AO), 2-phenylethyl acetate (PA) y furfuryl acetate (FA)) frente a *A, flavus* y *A. niger*, reduciendo también la producción de micotoxinas asociadas a estos mohos (Galván et al., 2022b). La aplicación de levaduras antagonistas durante la maduración del higo en el árbol podría reducir su contaminación fúngica y acumulación de micotoxinas. En cuanto a los microorganismos autorizados en España para el control de plagas de insectos de la higuera, se encuentra el hongo *Beauveria bassiana* y la bacteria *Bacillus thuringiensis.* (MAPA, 2023).

En el procesado del higo seco también tiene especial relevancia el control de agentes bióticos alterantes durante su procesado en la industria. En la Tabla 8 se muestran las diferentes prácticas culturales y de saneamiento de los almacenes, así como métodos de control de plagas y enfermedades del higo seco en la etapa postcosecha. Durante el procesado y almacenamiento de los higos secos en la industria es muy importante la vigilancia mediante inspección visual y colocación de trampas. Las larvas pueden verse en los frutos, en las paredes de las instalaciones o alrededor de envases. La mezcla de telarañas y excrementos son síntomas de una fuerte infestación (Turanlı, 2022).

Tabla 8. Métodos de prevención y control de plagas y enfermedades durante la etapa postcosecha en la industria.

Medidas de control		Descripción	Agente biótico	Referencia
Físicos	Prácticas para almacenado por el agricultor	- Almacenar frutos en cajas sin contacto con el suelo - Dejar espacio entre pilas de cajas y pared - Cerrar puertas y ventanas, cubrirlos con mosquiteras. - Paredes encaladas y sin grietas. - Lugar fresco y ventilado. - Sala y materiales utilizados limpios, incluso fumigados. - Eliminar frutos infestados - Evitar puntos de luz nocturnos - Evitar largos periodos de almacenamiento	**Insectos** **Mohos**	Turanlı (2022); FAO (1985); Codex Alimentarius (2008); Akol et al. (2020); Şen et al. (2009, 2010)
Biológicos	Atmósferas modificadas	- Ozono (O_3) - Dióxido de Carbono - Mezclas de gases (Nitrógeno CO_2 y oxigeno) - Tratamientos bajo presión	**Insectos** **Mohos**	Sadeghi et al. (2017); Öztekin et al. (2006); Şen et al., (2009, 2010); Aksoy et al. (2012); Damarh et al. (1998)
	Tratamiento térmico	- Radiación gamma - Microondas - Tratamiento de calor/frío - Almacenamiento en frío y humedad controlada	**Insectos** **Mohos**	Aziz and Moussa, (2002); Cetinkaya et al. (2006); Sadeghi et al. (2018); Guirguis (2018); Şen et al. (2010); Zare and Jalili (2020); Emekçi and Ferizli (2000)
	Trampas	- Cebo y pegajosas - Trampa de Luz UV - Feromonas	**Insectos**	Emekçi and Ferizli (2000); Karlis et al., 2008
	Polvos inertes	- Tierras diatomeas	**Insectos**	Murali, 2013
	Enemigos naturales	Parasitoides	**Insectos**	Johnson et al., 2000; Eliopoulos et al., 2004.
	Otros	Compuestos orgánicos volátiles	**Hongos**	Galván et al., 2022b
Químicos	Fumigación	- Fosfuro de aluminio - Fosfuro de magnesio	**Insectos**	Aksoy et al., 2008; Athanassiou et al., 2016; Ferizli et al., 2004; MAPA, 2023.

	Químicos naturales	- Extractos de plantas - Aceites esenciales	**Insectos** **Mohos**	Soltani et al., 2022; Satish et al., 2007; Pawar and Thaker., 2006; Jesser et al., 2017

Las instalaciones donde se van a almacenar deben de estar limpias e incluso en el caso que fuese necesario, fumigadas antes de la entrada de los higos secos. Debe ser un lugar fresco y ventilado, pero que no permita el acceso de insectos del exterior, por lo que puertas y ventanas deben estar cerradas o con mosquiteras. Evitar almacenar los frutos a granel o sacos, dejar espacio entre las cajas y la pared y transportar los higos a la industria para ser fumigados lo antes posible. Estas son algunas de las recomendaciones que deben seguir los agricultores para evitar la pérdida de calidad del fruto (FAO, 1985; Codex Alimentarius 2008; Şen et al., 2009, 2010; Akol et al., 2020; Turanlı, 2022).

Una vez los higos llegan a la industria, la forma más extendida para erradicar las plagas que vienen del campo es la fumigación. El bromuro de metilo era el pesticida más extendido para la desinfestación de productos agrícolas almacenados como frutos secos, cereales y frutas (Fields and White, 2002). Sin embargo, su uso como fumigante fue retirado a nivel mundial según la directiva del Protocolo de Montreal relativo a las sustancias que agotan la capa de ozono (Schneider et al., 2003). En su lugar, actualmente se utiliza el fosfuro de aluminio o magnesio, siendo los más extendidos y los únicos autorizados en España, con una licencia de uso que es necesario renovar en 2026 (MAPA, 2023). Los pesticidas autorizados son pocos y su uso debe restringirse debido al desarrollo de resistencia de las plagas, los peligros para la salud y el riesgo de contaminación medioambiental (Abd El-Aziz, 2011). Además, en este sentido tiene especial relevancia los objetivos del Pacto Verde de la UE comentados anteriormente. Por estas razones, en los últimos años se han realizado diversos estudios científicos buscando métodos

alternativos, que sean igual de efectivos en el control sin modificar la calidad del producto. Entre ellos, destacan por su eficacia, la aplicación de ozono, dióxido de carbono, uso de atmósferas modificadas junto a altas temperaturas, uso de radiación gamma o microondas (Damarh et al., 1998; Cetinkaya et al., 2006; Öztekin et al., 2006; Şen et al., 2009, 2010; Aksoy et al., 2012; Sadeghi et al., 2017; Sadeghi et al., 2018). Şen et al., (2009) observaron que la aplicación de CO_2 controlaba eficazmente las plagas de almacenamiento sin efectos importantes sobre la calidad de los frutos y que la aplicación en tanques presurizados reducía la duración del tratamiento. Igualmente, para otros autores la aplicación de ozono ha mostrado una gran eficacia en el control de las plagas *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) y *Ephestia kuehniella* (Lepidoptera: Pyralidae) (Sadeghi et al., 2017). Por otro lado, Öztekin et al., (2006) tras aplicar ozono a higos secos, obtuvieron una reducción estadísticamente significativa de recuentos totales de bacterias, coliformes y levaduras/mohos. En el mismo sentido, Zorlugenç et al. (2008) determinaron que la aplicación de ozono o de agua ozonizada durante 15 minutos fue suficiente para la inactivación de todos los mohos, con una degradación de la AFB1 a medida que incrementaba el tiempo de ozonización. En frutos secos como el dátil, de composición nutricional similar al higo seco, se han realizado varios estudios sobre tratamientos de congelación para el control de *E.ceratoniae,* obteniéndose buenos resultados. Se alcanzó el 100% de la mortalidad de esta especie y no se observaron diferencias entre los higos congelados y sin tratar (Ben-Amor et al., 2019; Jemni et al., 2019).

Todos estos tratamientos pueden controlar las infestaciones existentes, pero no protegerán de la reinfestación, por lo que deben tomarse todas las precauciones necesarias (Akol et al., 2020). Para ello, al igual que durante su exposición al aire libre, uno de los

métodos utilizados son los trampeos, siendo las trampas de luz ultravioleta o trampas pegajosas para polillas las más utilizadas en la industria.

También es importante controlar las contaminaciones fúngicas que contienen los higos secos en esta etapa, evitando proporcionar las condiciones de temperatura y humedad necesarias para su propagación y producción de micotoxinas. Algunas de las medidas adecuadas para evitarlo, consisten en el secado y enfriamiento rápido de los higos después del escaldado, junto con el almacenamiento en todo momento en condiciones de refrigeración y humedad relativa controlada (Galván, 2022). En cuanto a métodos de control biológicos, tal y como se indicó anteriormente, Galván et al. (2022b) demostraron que VOCs como acetato 2-feniletilo y acetato de furfurilo controlaron eficazmente el crecimiento de *A. flavus* y *A. niger* y la producción de AF y OTA. El uso de estos VOCs en la etapa postcosecha durante el almacenamiento o empaquetado activo podría ser eficiente en el control de la proliferación de estos mohos toxigénicos.

La búsqueda de estrategias o metodologías de control de plagas y enfermedades en toda la cadena de producción del higo seco, sostenibles con el medio ambiente y seguras para la salud de los consumidores y que garanticen la calidad higiénico-sanitaria del higo seco preservando su calidad nutricional y sensorial, es una de las principales problemáticas actuales del sector. En este sentido, tal y como se ha puesto de manifiesto anteriormente, la comunidad científica está realizando un notable esfuerzo en la búsqueda de estrategias o metodologías alternativas, aunque aún son limitadas las propuestas que se están aplicando en el sector de gran importancia socioeconómica para Extremadura. Por lo tanto, las actividades de I+D+i deben fortalecerse para conseguir los objetivos planteados por las políticas de la UE en el sector agroalimentario, en este caso del higo seco.

OBJETIVOS

OBJETIVOS/OBJECTIVES

El objetivo general de esta Tesis Doctoral fue cuantificar e identificar las plagas y enfermedades que afectan al higo seco en las principales zonas de producción de Extremadura, así como establecer y evaluar métodos de control durante todo el proceso productivo que permitan minimizar los daños y obtener un producto de excelente calidad higiénico-sanitaria. Para avanzar en la consecución de dicho objetivo general se plantearon los siguientes objetivos específicos:

The general objective of this Doctoral Thesis was to quantify and identify the pests and diseases that affect dried figs in the main production areas of Extremadura, as well as to establish and evaluate control methods throughout the production process to minimize damage and obtain a product of excellent hygienic-sanitary quality. The following specific objectives were proposed to advance in the achievement of this general objective:

1. Analizar los daños bióticos en higos secos producidos en Extremadura en base al grado de manejo y régimen hídrico de los higuerales, así como identificar las especies de insectos y microbiota fúngica responsables de los mismos.

To analyze the biotic damage to dried figs produced in Extremadura based on the degree of management and water regime of the fig orchards, as well as to identify the insect species and mycobiota responsible for this damage.

2. Evaluar la eficacia de un sistema de mallas adaptado a la copa de la higuera para proteger al higo durante su desarrollo, secado y recolección frente agentes bióticos presentes en el entorno.

Evaluation of the effectiveness of a netting system adapted to the fig tree canopy to protect figs during development, drying and harvesting from biotic agents present in the environment.

3. Establecer la congelación como alternativa a la fumigación con fosfuro de aluminio de los higos secos, optimizando la combinación de diferentes tiempos y temperaturas para la desinfestación total, así como los efectos de estos tratamientos sobre la calidad fisicoquímica, microbiológica y sensorial del producto.

To establish freezing as an alternative to aluminium phosphide fumigation of dried figs, optimizing the combination of different times and temperatures for total disinfestation, as well as the effects of these treatments on the product's physicochemical, microbiological and sensory quality.

4. Evaluar la eficacia de mallas impregnadas en alfacipermetrina para el control de larvas de plagas de almacén y así prevenir reinfestaciones en higos secos durante su almacenamiento en la industria.

To evaluate the efficacy of alphacypermethrin-impregnated nets for controlling storage pest larvae to prevent reinfestations in dried figs during storage in the industry.

DISEÑO EXPERIMENTAL GENERAL

DISEÑO EXPERIMENTAL GENERAL

Esta Tesis Doctoral se estructura en cuatro capítulos, titulados:

CAPÍTULO 1. Cuantificación e identificación de daños causados por plagas y hongos en higos secos procedentes de huertas con diferentes niveles de manejo agronómico en las principales zonas productoras de Extremadura.

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.

CAPÍTULO 2. Implantación del sistema de malla Witty® para la producción de higos secos 'Calabacita': efectos sobre la incidencia de plagas, la calidad del fruto y la presencia de micotoxinas.

Implementation of Witty® net system for production of 'Calabacita' dried figs: effects on pest incidence, fruit quality and mycotoxin occurrence.

CAPÍTULO 3. Tratamiento de congelación como alternativa al control convencional de plagas en higos secos y su efecto sobre la calidad global del fruto

Freezing treatments as an alternative to conventional pest control in dried figs and their effect on global fruit quality.

CAPÍTULO 4. Evaluación de Carifend® para el control de larvas de las principales especies de productos almacenados.

Evaluation of Carifend® for the control of larvae of major stored-product species.

En la Figura 34 se muestra un resumen ilustrado de los objetivos y del diseño experimental general de los cuatro capítulos que componen este trabajo.

Esta tesis está enmarcada dentro del proyecto de investigación RTA2017-00032-C02-01, denominado "Gestión integral del proceso productivo del higo seco que asegure un producto de máxima calidad higiénico-sanitaria". Y a su vez, financiada por la beca predoctoral: PRE2018-086475, del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Centro Nacional integrado en la Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC) perteneciente al Ministerio de Ciencia e Innovación.

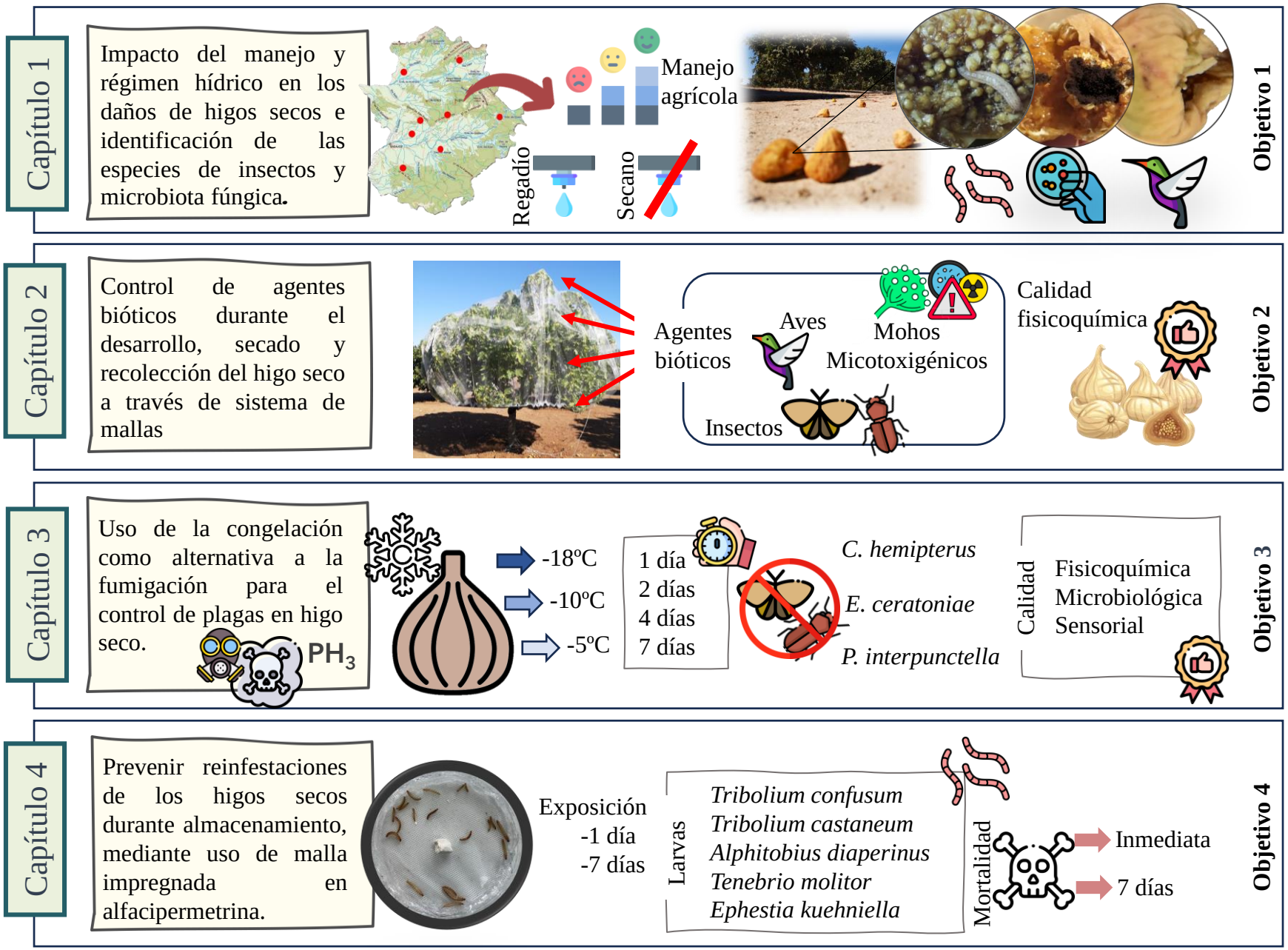


Figura 34. Diseño experimental general.

CHAPTER 1

CHAPTER 1

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)

1. 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.

Keywords: Dried figs, Pests, Birds, Insects, Moulds, Agronomic management.

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 ºC; winters can be mild and rainy or cold and dry (Moral et al., 2016). Nine of the 18 orchards were in irrigated plantations, and the other nine were in rainfed plantations. In the irrigated plantations, 2,500–3,000 m^3/ha 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 1.1. The dried fig sampling, inspections and determinations performed are graphically presented in Figure 1.1. Specifically, in each orchard, one kilogram 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 metres from each other. A total of 180 samples were collected (5 samples × 18 orchards × 2 seasons). The samples were transported under refrigeration to the laboratory for analysis.

Table 1.1. Description of the agronomic management and specific location of the 18 fig orchards sampling in Extremadura (SW Spain)

	Nº	Location	Density (number of trees/ ha)	[1]W. Station	Crop Management
Rainfed	1	38°31'01.6"N 6°52'08.8"W	277	BA05	**HIGH** - Bordeaux mixture treatments in winter - Annual pruning -[2]Nutrient control and fertilisation plan - Weeding and preparing the soil before fruit fall - One harvest per week - Various bird repellents (hunting, sound cannons, visual bird deterrents, et cetera)
	2	39°03'02.2"N 6°32'07.5"W	204	BA205	
	3	38°52'14.2"N 6°03'30.6"W	74	BA04	
Irrigated	4	39°09'05.8"N 6°01'58.4"W	156	BA106	
	5	39°00'39.9"N 6°03'38.6"W	400	BA106	
	6	39°08'58"N 6°01'58.1"W	156	BA106	
Rainfed	7	39°22'21.1"N 5°23'31.5"W	156	CC103	**MEDIUM** - Pruning every two or three years -[3]Unplanned fertilisation - Weeding and preparing the soil before fruit fall - Two harvests per season - Some methods of bird repellent (sound or visual deterrents)
	8	39°49'46.9"N 6°47'06.3"W	277	CC104	
	9	39°09'32.7"N 6°02'04.7"W	100	BA106	
Irrigated	10	40°03'23.3"N 5°44'20.7"W	400	CC10	
	11	40°03'13.0"N 5°44'15.1"W	400	CC10	
	12	39°16'34"N 5°42'48.9"W	1900	CC07	
Rainfed	13	40°03'18.5"N 5°53'06.2"W	277	CC10	**LOW** - No pruning - No fertilisation - One harvest at the end of the season - No bird repellents
	14	39°09'25.4"N 6°02'06.2"W	100	BA106	
	15	39°02'30.9"N 6°31'24.0"W	204	BA205	
Irrigated	16	40°02'57.6"N 6°40'25.7"W	625	CC16	
	17	38°29'00.3"N 6°51'11.8"W	70	BA05	
	18	38°51'48.6"N 6°39'59.3"W	400	BA205	

[1] The nearest weather station to the location where humidity, temperature and rainfall data were recorded in REDAREX, 2023

[2]Winter fertilisation (Dec) with ~300 kg/ha NPK (9:18:27) and spring (May) fertilisation with ~100 kg/ha potassium nitrate. Soil nutrient analysis every 4-5 years

[3] Only winter fertilisation (Dec) with ~300 kg/ha NPK (9:18:27)

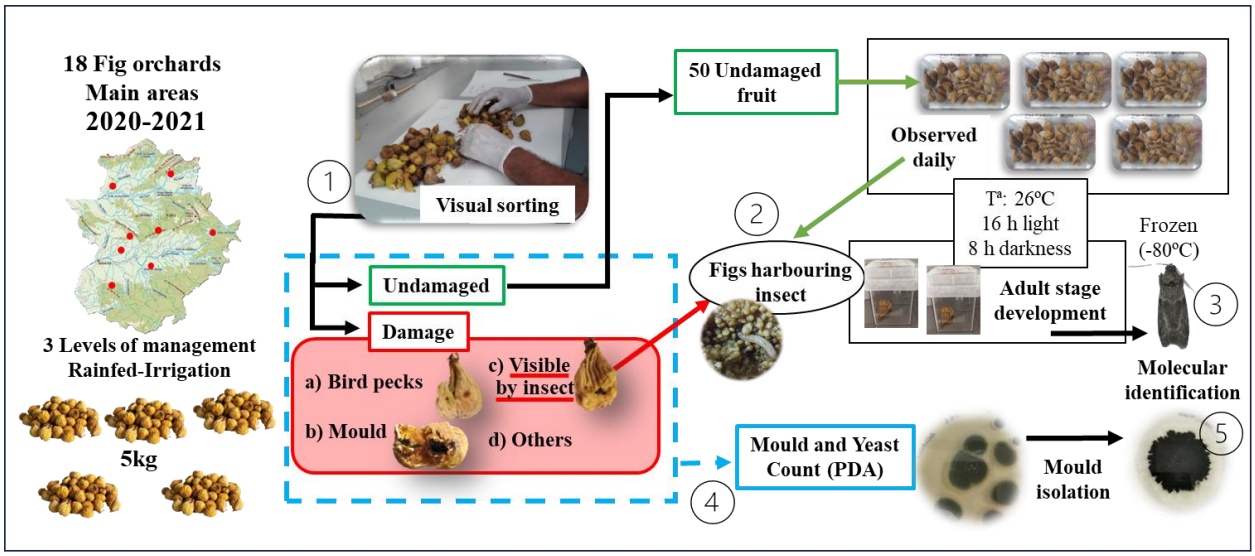


Figure 1.1. Plant material and experimental design; the red dots on the map of the Autonomous Community of Extremadura (Spain) indicate the sampling locations.

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 ± 1 ºC 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 ºC. 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 ± 1 ºC 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 × % infestation fruits from visible insect damage category) + (% fruits from the category: undamaged × % 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'-GGTCAACAAATCATAAAGATATTGG-3)' and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). 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× 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: 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 grams 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 seconds. 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 at 25 °C for 7 days, and then a portion of approximately 0.5 cm^2 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'-CTTGGTCATTTAGAGGAAGTAA-3; ITS4: 5'-TCCTCCGCTTATTGATATGC-3'; White et al., 1990) and Bt2a/Bt2b (Bt2a: 5'-GGTAACCAAATCGGTGCTGCTTTC-3'; Bt2b: 5-ACCCTCAGTGTAGTGACCCTTGGC-3'; Glass and Donaldson, 1995), 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 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C 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 (three-way 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 1.2. 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 1.3). 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 (Şen, 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). 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 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) found bird damage between 11 and 30% of fresh fig and breba crops (fruit set on wood from the previous year), while Galván et al. (2021) attributed between 29.3 and 55.7% of all damage recorded in dried figs to birds.

Table 1.2. ANOVA parameters for main effects and associated interaction for percentage of damage fruits and infested dried figs with insects.

Source	n	df	*Damage fruit*								*Infested with insect*					
			Undamaged		Bird pecks		Visib. insect damage		Mould		Others		Undamaged		Visib. insect damage		Total estimate	
			F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
All between																		
Intercept	180	1	7991.5	<0.001	267.4	<0.001	291	<0.001	48.2	<0.001	278.4	<0.001	129.7	<0.001	143.8	<0.001	146.0	<0.001
Water regime	90	1	5	0.026	8.4	0.004	0.1	0.821	0.6	0.459	0.5	0.499	0.8	0.374	14.5	<0.001	2.5	0.118
Season	90	1	14	<0.001	5.8	0.017	5.2	0.024	1.3	0.252	10.9	0.001	0.0	0.843	7.0	0.009	0.1	0.705
Crop management	60	2	33.4	<0.001	52.1	<0.001	8.8	<0.001	0.6	0.571	7.1	0.001	1	0.375	15.5	<0.001	0.9	0.416
Water regime x Crop management	180	2	30.6	<0.001	37.2	<0.001	13	<0.001	4.1	0.016	3.3	0.039	2.2	0.113	24.1	<0.001	6.1	0.003
Water regime x Season	180	1	0.3	0.592	1.1	0.289	0.9	0.337	2.9	0.092	0.8	0.365	4.7	0.032	0.2	0.648	5.2	0.023
Crop management x Season	180	2	1.6	0.205	2.9	0.058	0.3	0.724	1.7	0.180	3.9	0.022	0.7	0.501	0.1	0.887	0.0	0.974
Water regime x Crop management x Season	180	2	2.1	0.120	0.9	0.394	1.1	0.337	0.2	0.788	1.7	0.192	9.5	<0.001	0.5	0.587	5.0	0.008
Error		168																

Table 1.3. Mean percentages ± SD of undamaged and damaged fruit relative to the water and crop management regime

		Rainfed				Irrigated			
		High	**Medium**	**Low**	**Mean Rainfed**	**High**	**Medium**	**Low**	**Mean Irrigated**
Undamaged		79.63 ± 12.77[2]	81.24 ± 10.34	79.22 ± 20.27[1]	80.03 ± 14.92	91.34 ± 5.58[a1]	79.35 ± 9.49[b]	57.65 ± 10.04[c2]	76.11 ± 16.39
Damaged	Bird pecks	9.50 ± 8.06[1]	8.55 ± 8.37	11.72 ± 17.13[2]	9.92 ± 11.89	1.13 ± 1.63[c2]	8.04 ± 7.84[b]	33.39 ± 11.09[a1]	14.18 ± 15.98
	Insect	5.09 ± 4.96[1]	4.39 ± 3.63[2]	5.81 ± 4.68	5.10 ± 4.45	1.81 ± 2.27[c2]	8.61 ± 4.69[a1]	5.28 ± 3.70[b]	5.23 ± 4.59
	Mould	0.60 ± 0.85	0.79 ± 1.32	1.21 ± 1.92[1]	0.87 ± 1.44	1.28 ± 2.44[a]	0.51 ± 1.27[ab]	0.30 ± 0.43[b2]	0.70 ± 1.64
	Others	5.20 ± 4.22[a]	5.03 ± 4.33[a]	2.05 ± 1.51[b]	4.09 ± 3.84	4.44 ± 3.04	3.49 ± 3.68	3.38 ± 2.03	3.77 ± 3

[a,b,c] Indicate significant differences $p \leq 0.05$ between the crop management levels with the same water management regime

[1,2] Indicate significant differences $p \leq 0.05$ between water management regimes with the same level of crop management

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). 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). The most widespread and effective method in fig crop is the use of a physical barrier such as nets (Singh et al., 2022). 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; Tracey et al., 2007; Simon, 2008). 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), 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 1.1), 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), who found in the Extremadura production area mould damage in 1.64 to 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 ($p \leq 0.05$). 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 1.4). 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 mold) showed significant differences ($p \leq 0.05$).

Table 1.4. Mean percentages of damage and insect infested fruit relative to water regime and crop management level by season,

Variable	Rainfed						Irrigation					
	High		Medium		Low		High		Medium		Low	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Damage category												
Undamage	79.15	80.1	78.3	84.2	71.1	87.32*	89.2	93.5	74.8	83.90*	55.61	59.69
Bird pecks	9.18	9.81	10.3	6.79	17.96*	5.48	0.85	1.4	9.47	6.61	35.22	31.56
Visib. insect damage	5.83	4.34	3.93	4.85	6.71	4.9	3.06	0.57	9.87	7.35	5.71	4.85
Molds	0.66	0.54	1.03	0.55	1.87*	0.55	0.95	1.62	0.57	0.46	0.4	0.21
Others	5.18	5.21	6.44*	3.62	2.34	1.75	5.92*	2.96	5.3*	1.69	3.07	3.69
Infested with insect												
Undamage	2.44	6.44*	0.89	5.78*	5.78	2.22	3.56	2.22	7.56*	2.44	4.89	6.89
Visib. insect damage	32.6	21.92	12.22	5.37	10.33	4.59	23.69	18.89	12.26	1.87	64.57*	46.75
Total estimate	4.54	5.99	0.82	4.88*	3.72	2.24	3.85	2.31	6.94*	2.25	6.43	7.04

*Indicate significant differences $p \leq 0.05$ between the seasons with the same water regime and crop management

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 1.5). 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, 2004; UNECE, 2016). 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), such as ants (Burks and Brandl, 2005) or the coleopteran *Gonocephalum pusillum* Fabricius (Coleoptera: Tenebrionidae) (Gragera-Facundo, 2014), that cause superficial damage to dry figs without infesting them.

Table 1.5. Mean percentages ± SD of undamaged and damaged fruits infested with insects relative to the water and crop management regime

	Rainfed				**Irrigated**			
	High	Medium	Low	Mean Rainfed	High	Medium	Low	Mean Irrigated
Undamaged	4.44 ± 5.76	3.33 ± 3.91	4 ± 6.03	3.93 ± 5.28	2.89 ± 4.35	5 ± 6.24	5.89 ± 5	4.59 ± 5.35
Visible Insect-damaged	27.26 ± 25.71[a]	8.79 ± 21.38[b]	7.46 ± 11.74[b2]	14.50 ± 22.18[2]	21.29 ± 30.63[b]	7.07 ± 15.42[b]	55.66 ± 31.77[a1]	28.02 ± 33.66[1]
Total estimate	5.26 ± 5.86	2.85 ± 3.15	2.98 ± 3.51[2]	3.70 ± 4.44	3.08 ± 4.2[a]	4.59 ± 6.46[ab]	6.7 ± 4.98[b1]	4.80 ± 5.45

[a,b,c] Indicate significant differences $p \leq 0.05$ between the crop management levels with the same water management regime

[1,2] Indicate significant differences $p \leq 0.05$ between water management regimes with the same level of crop management

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 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 ($p \leq 0.05$) in both water regimes at a medium management level (Table 1.4). 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). The average daily relative humidity (RH) and temperature were 42.9% and 26.1ºC during July and August of both years studied (REDAREX, 2023). However, irrigation in crops affects the microclimate of the environment by increasing humidity and reducing the temperature (Rosenberg, 1974; Sun et al., 2022). 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 and Hafez, 1979; James and Vogele, 2000; Cox, 1974). In addition, irrigation promotes the emergence of weeds (Verma et al., 2015), which can become a reservoir of insect pests and diseases in orchards where weeds are not controlled (low management) (Kumar et al., 2021). 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), 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) 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 1.6). A smaller number of insects that could not be identified by DNA barcoding were classed as “other species”.

Table 1.6. Relative abundance (%) of insect species in dried figs from different water and crop management regimes

Insect species	BOLD Barcode Index Numbers	Rainfed			Rainfed Total	Irrigated			Irrigated Total
		High	Medium	Low		High	Medium	Low	
Cadra abstersella	BOLD:AAW5130	5.1	47.2	-	10.1	4.6	6.8	14.7	10.8
Cadra figulilella	BOLD:AAZ9283	29.2	27.8	78.9	44.5	62.8	9.1	25.6	26.1
Ectomyelois ceratoniae	BOLD:AAU4812	9.5	11.1	5.6	8.8	7.0	10.2	5.8	7.3
Ephestia Parasitella	BOLD:AAD1430	0.7	-	-	0.4	-	-	1.9	1.1
Cryptoblabes gnidiella	BOLD:AAW5129	-	-	-	-	-	-	8.3	4.5
Carpophilus hemipterus	BOLD:AAN6006	5.8	-	1.4	1.3	4.7	65.9	21.1	32.4
Ceratitis capitata	BOLD:AAA3297	46.7	-	2.8	27.7	11.6	-	19.9	12.5
Drosophila simulans	BOLD:AAE8098	-	-	-	-	7.0	1.1	-	1.4
Venturia canescens	BOLD:AAH1679	-	-	1.4	0.4	2.3	-	2.6	1.7
Others		2.9	13.9	9.9	6.7	-	6.8	-	2.1

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), 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. (1932) and Jalili et al. (2004) 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) 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* (Millière) (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; Elnagar, 2018), and *C. gnidiella* has been even found damaging acorns (Torres-Vila et al., 2002).

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). 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) 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), 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).

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). 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., 2022). 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 Figure 1.2.

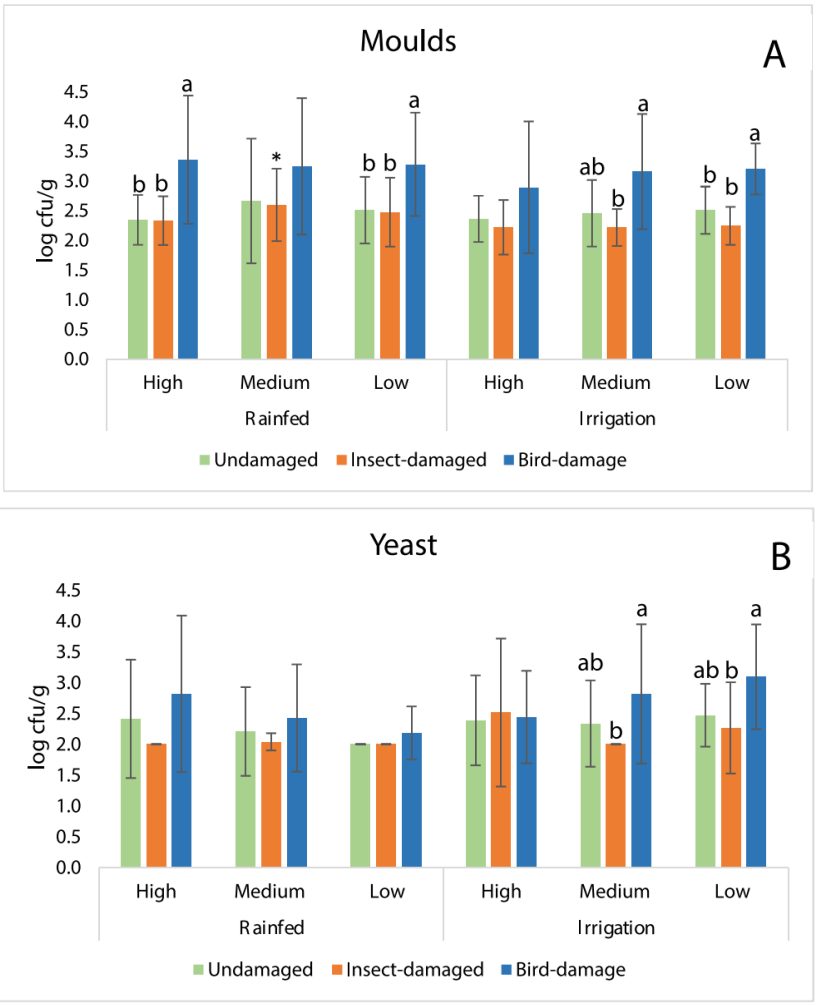


Figure 1.2 Mean counts ± SD (log cfu/g) of moulds (A) and yeasts (B) in dried figs classified as undamaged (green), insect-damaged (orange) and bird-damaged (blue) from different levels of crop management and water regime. a,b,c Indicate significant differences $p \leq 0.05$ between undamaged fruit and 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.

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) and Galván et al. (2023, 2022a) 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). 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), 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., 2012). 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 cfu/g, respectively. In contrast, figs damaged by bird pecks had the highest 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), 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; Galván et al., 2022a). 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

1.7), 89.47% of which belonged to the genera *Aspergillus*, *Alternaria*, *Penicillium*, *Cladosporium*, *Talaromyces* and *Fusarium*. Table 1.8 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). 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). A total of 16 species of this genus were identified, with a high prevalence of *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; Galván et al., 2023, 2022a).

Table 1.7. Percentage of mold species identified by gene marker sequencing.

Identification by ITS and β-tubulin sequencing	**GenBank accession number ITS**	**GenBank accession number β-tubulin**	**%**	**Code**
Alternaria sect. Alternaria	MT174142.1	KY814627.1	4.5	AA
Alternaria sect. Infectoriae	MK460886.1	KY965830.1	0.5	AI
Alternaria sect. Ulocladioides	MK713361.1	-	4.5	AU
Alternaria eureka	MH399493.1	-	0.5	AE
Aspergillus alliaceus	MH865237.1	MG517764.1	0.5	AAI
Aspergillus amstelodami	MG655303.1	MK361163.1	0.5	AAm
Aspergillus chevalieri	MT316337.1	KU872178.1	0.5	ACh
Aspergillus europaeus	FJ531070.1	MT452519.1	0.5	AEu
Aspergillus flavus	MH864264.1	CP059871.1	6	AFl
Aspergillus fumigatus	MH865793.1	KU935623.1	0.5	AFu
Aspergillus ibericus	NR_119514.1	MH614574.1	0.5	AIb
Aspergillus melleus	MH864931.1	MT410177.1	1.5	Ame
Aspergillus niger	MT447518.1	LC387870.1	6.5	Ani
Aspergillus parasiticus	MT079321.1	KY416555.1	1.5	Apa

Aspergillus tamarii	MH865259.1	EF661474	2.5	Ata
Aspergillus terreus	NR_131276.1	MK159784.1	0.5	Ate
Aspergillus tubingensis	LC573618.1	LC387873.1	21.5	ATu
Aspergillus udagawae	MT530099.1	KY808586.1	1	AUd
Aspergillus uvarum	OL711726.1	MK854752.1	0.5	AUv
Aspergillus welwitschiae	MH374611.1	MG832179.1	24.5	AWe
Cladosporium halotolerans	MT529175.1	-	1	CHa
Cladosporium oxysporum	NR_152267.1	MF175220.1	2	COx
Fusarium oxysporum	MK817045.1	MN646768.1	1	FOx
Penicillium bilaiae	KU872788.1	MK451244.1	1	PBi
Penicillium brevicompactum	MT558924.1	MK682838.1	1	PBr
Penicillium citrinum	MH858380.1	GU944545.1	1	PCi
Penicillium crustosum	MT316358.1	AY674351.1	1.5	PCr
Penicillium expansum	MH578582.1	AY674399.1	2	PEx
Penicillium hispanicum	MT294667.1	JF521519.1	0.5	PHi
Penicillium melanoconidium	NR_160219.1	JX091545.1	0.5	PMe
Penicillium menonorum	MT529078.1	MK682868.1	0.5	PMe
Penicillium sizovae	MH859338.1	GU944536.1	0.5	PSi
Talaromyces amestolkiae	MT441607.1	MH287207.1	1	TAm
Talaromyces pinophilus	MF589639.1	MK451196.1	0.5	TPi
Talaromyces purpurogenus	AB872819.1	KC345004.1	0.5	TPu
Talaromyces trachyspermus	MT528783.1	MK451251.1	0.5	TTr
Boeremia exigua	MK907733.1	MK907733.1	0.5	O
Didymella americana	KY070283.1	MK032765.1	0.5	O
Dothiora viticola	MK782424.1	-	0.5	O
Nothophoma quercina	MK088575.1	KY053899.1	0.5	O
Paraconiothyrium brasiliense	JF439492.1	JX496397.1	0.5	O
Periconia macrospinosa	OK356521.1	-	0.5	O
Pseudothielavia arxii	MZ724891.1	MK926931.1	1.5	O
Rhizopus arrhizus	MT316366.1	-	0.5	O
Stagonosporopsis cucurbitacearum	OP020692.1	-	0.5	O
Trichoderma gamsii	MK361138.1	-	0.5	O

Table 1.8. Relative abundance (%) of mould genera on damaged and undamaged dried figs from different management levels

Water management regime	Crop management level and damage category	*Aspergillus* spp.	*Penicillium* spp.	*Alternaria* spp.	*Cladosporium* spp.	*Talaromyces* spp.	Others
Rainfed	High	80	8.6	2.9	0^{b}	0	8.6
	Medium	73.2	9.8	2.4	0^{b}	2.4	12.2
	Low	67.6	8.1	5.4	8.1^{a}	0	10.8
	Undamaged	71.4^{2}	14.3^{12}	14.3^{1}	0^{2}	0^{2}	0^{2}
	Bird pecks	50^{2}	20^{1}	5^{2}	0^{2}	5^{1}	20^{1}
	Mould	93.8^{1}	4.2^{3}	0^{3}	0^{2}	0^{2}	2.1^{2}
	Insect	58.1^{2}	6.5^{23}	3.2^{2}	9.7^{1}	0^{2}	22.6^{1}
	Total	**73.5***	**8.8**	**3.5**	**2.7**	**0.9**	**10.6**
Irrigated	High	75^{a}	2.8^{b}	8.3^{b}	0^{b}	5.6^{a}	8.3^{b}
	Medium	56.8^{b}	5.4^{b}	24.3^{a}	0^{b}	0^{b}	13.5^{ab}
	Low	26.1^{c}	17.4^{a}	17.4^{ab}	13^{a}	8.7^{a}	17.4^{a}
	Undamaged	11.1^{3}	0^{3}	44.4^{1}	11.1^{1}	22.2^{1}	11.1^{2}
	Bird pecks	34.8^{2}	4.3^{2}	$13^{2,3}$	8.7^{1}	8.7^{1}	30.4^{1}
	Mould	86^{1}	4.7^{2}	7^{3}	0^{2}	0^{2}	2.3^{3}
	Insect	38.1^{2}	19^{1}	$28.6^{1,2}$	0^{2}	0^{2}	14.3^{2}
	Total	**56.3**	**7.3**	**16.6***	**3.1**	**4.2***	**12.5**

[a,b,c] Indicate significant differences $p \leq 0.05$ between the crop management levels with the same water management regime

[1,2,3] Indicate significant differences $p \leq 0.05$ between undamaged fruit and different types of fruit damage with the same water management regime

*Indicates significant differences $p \leq 0.05$ between water management regimes

In contrast, the incidence of *Alternaria* spp. was significantly higher ($p \leq 0.05$) in figs produced in irrigated orchards than in rainfed orchards, at 16.6% and 3.5% respectively. Galván et al., (2023) 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., 2015b). 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), 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 mycotoxin-producing species associated with dried figs (Taniwaki et al., 2018). 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; Can, 2022).

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). 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). However, birds and insects can be vectors of entry by transmitting the spores of these fungi. Phillips et al. (1925) showed that the pests *C. hemipterus* and *Drosophila melanogaster* can transmit fungal spores to edible figs, and Michailides (1991) 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). These findings are consistent with those recorded in California by Doster et al. (1996), 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 (Şen, 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; Mat Desa et al., 2019).

The PCA loadings and scoring plots summarise the identified mould species according to the crop management level and type of damage (Figure 1.3). 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. 1.3 A); this behaviour is explained by the negative loading on the same axis (Fig. 1.3 B) 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. 1.3A), which is attributed to the higher presence of *Alternaria* spp. It can also be seen that samples from rainfed 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. 1.3A) 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. 1.3A), while *Aspergillus welwitschiae* and *Aspergillus niger* are located on the negative side of the PC1 axis (Fig.1.3 B).

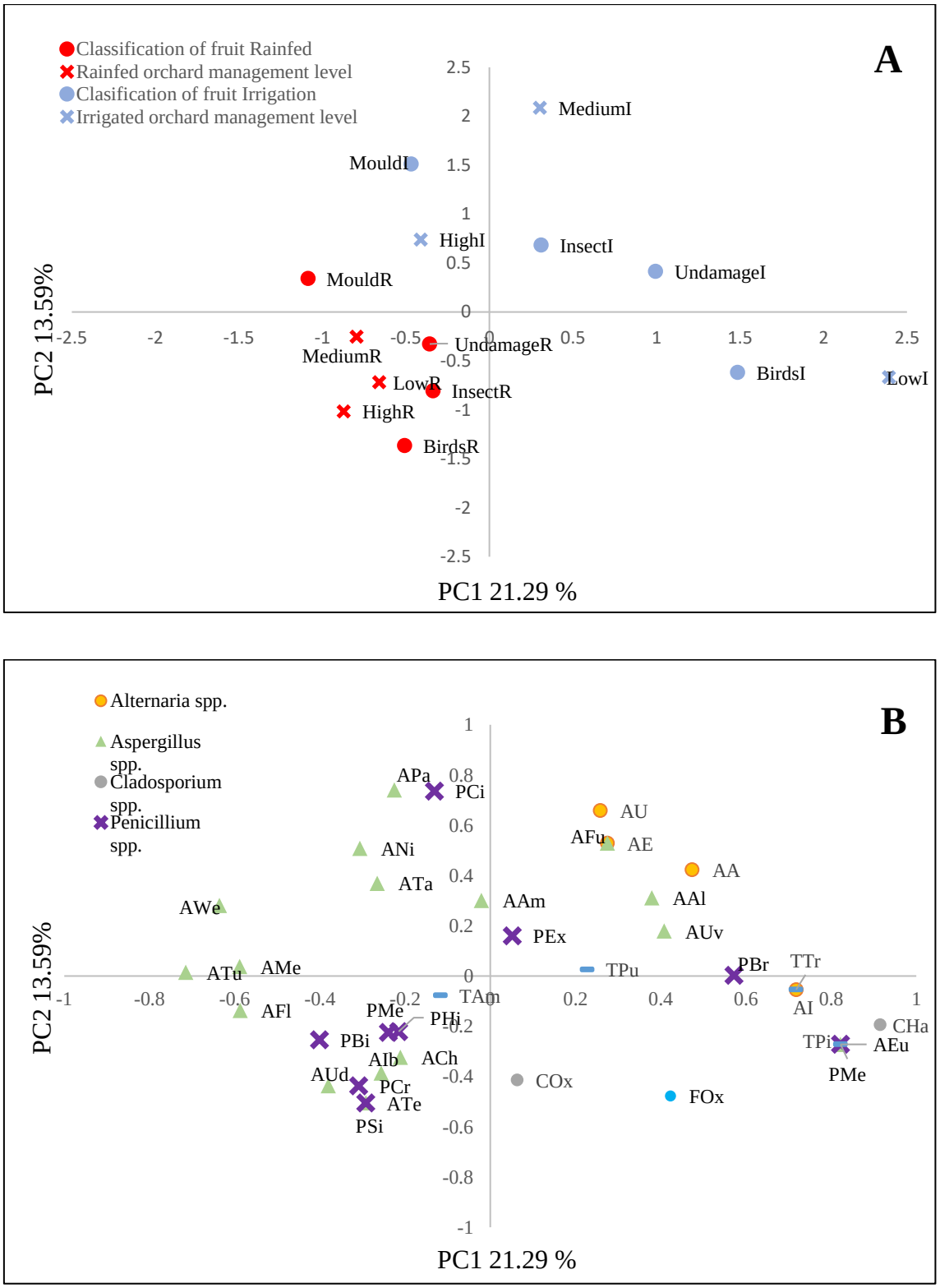


Figure 1.3. Projection of samples grouped according to water regime, crop management and classification of fruits (A) and mould species identified (B) in the space defined by the first two components (PC1/PC2): Alternaria eureka (AE), Alternaria section Alternaria (AA), Alternaria section Infectoriae (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)

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.

CHAPTER 2

CHAPTER 2

Implementation of Witty® net system for production of 'Calabacita' dried figs: effects on pest incidence, fruit quality and mycotoxin occurrence.

1. Abstract

This work aimed to evaluate the effect of using Witty® net system on 'Calabacita' fig variety during harvesting period on fruit damage, physicochemical and microbiological quality, and mycotoxins occurrence. For this purpose, dried fig samples were collected from three different orchards located in Extremadura (Spain) production area, selecting 12 trees with similar characteristics in each orchard. Before harvesting, the Witty® net system was placed in six fig trees in each orchard, while in the remaining six trees, the traditional management was followed. The results showed that the use of Witty® net led to a considerable reduction in the percentage of damaged fruit caused by birds and Lepidoptera insects, whereas fruit damage by Coleoptera remained unchanged. The efficiency in the control of fruit damage caused by birds was notably, decreasing from 11.93% to 1.02% in comparison with traditional system. In addition, Witty® net system modified positively physicochemical parameters that play a decisive role in sensory quality. Dried figs obtained with the Witty® system had significant greater fruit weight, softer, firmness and lighter reddish brown colour. In contrast, it did not influence the microbial counts of moulds, However, outstanding, its use significantly reduced the occurence of most of the mycotoxins detected, leading to higher percentage of samples below the limit of detection for aflatoxins B2 and G2 (AFG2), kojic acid and alternariol (AOH). Moreover, we found lower percentage of samples contaminated at level ranging from 8 to 40 µg/kg for ochratoxin B, AFG2 and AOH and at level >40 µg/kg for ochratoxin A, aflatoxins B1, and G1 and kojic Acid. Therefore, although the installation

of Witty® net involves a initial cost for farmers, our findings demonstrate its effectiveness in reducing pest incidences and provide dried fig with better physicochemical and hygienic-sanitary quality. These scientific evidences together with the improvement that the Witty® net brings to harvesting operations suggest that its use could lead to more profitable production and also positively influence the marketability of dried figs.

Keywords: Dried figs, Pest control, Fruit quality, mycotoxin, crop management.

2. Materials and methods

2.1- Experimental design

The experimental design is showed graphically in Figure 2.1. To evaluate the impact of Witty® net (WLargro, Cáceres, Spain) in dried fig quality, 12 fig trees by orchard of homogeneous size and age were selected in three commercial orchards of variety 'Calabacita' located in the representative production areas Guadajira, La Nava de Santiago and Arroyomolinos of the Autonomous Community of Extremadura in the season 2021.

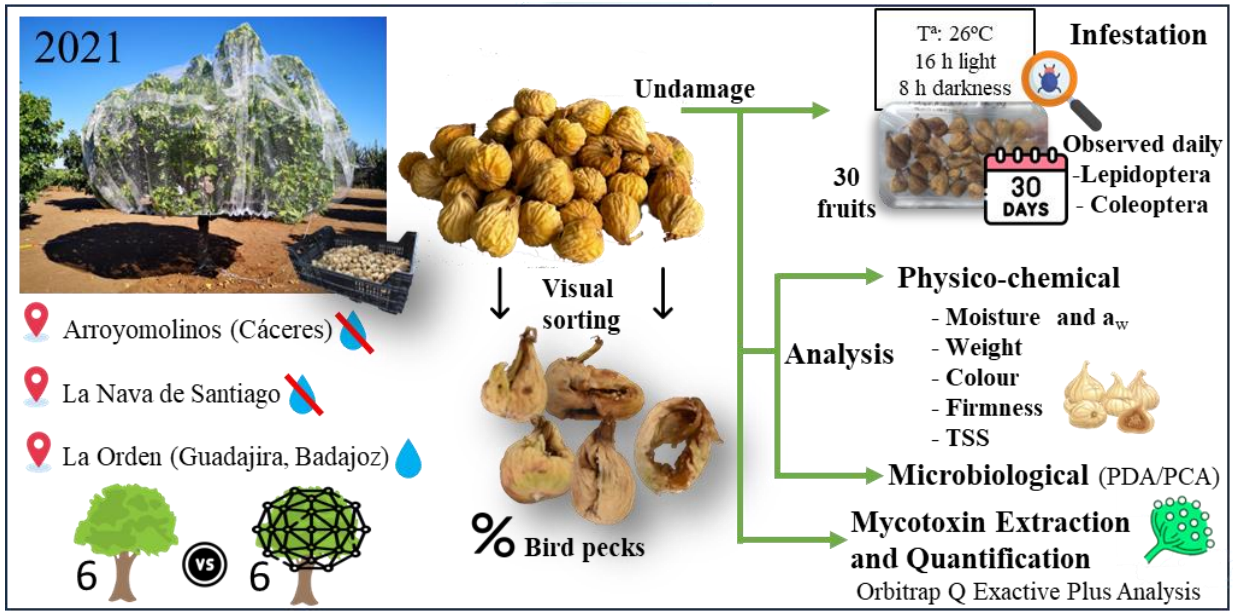


Figure 2.1. Plant material and experimental design.

The main agronomic characteristics of each orchard are shown in Table 2.1. In each orchard, Witty® net was installed on 6 fig trees approximately 15 days before the figs reached ripening to the end of harvest (Figure 2.2), whereas the production of dried figs from the other six fig trees was used as a control. The Witty® system is made up of a white upper leno weave net of polyethylene monofilament (cell size 7 x 3 mm) with weighs of 40 g/m^2, which provides between 15 and 18% shade to the tree, and a suspended black bottom net of 100% polyester (cell size 2 x 2 mm) for harvesting the dried figs, avoiding their contact with the ground (Figure 2.2).

Table 2.1. Geolocation and the main agronomic fig crop management characteristics applied in the three orchards evaluated.

Location	Coordinates	Density (number of trees/ ha)	Crop conditions
Arroyomolinos (Cáceres)	39°11'43.4"N 6°10'23.6"W	83	- Rainfed - Diameter of the tree canopy 6 m
La Nava de Santiago (Cáceres)	39°03'09.9"N 6°32'26.3"W	204	- Rainfed - Diameter of the tree canopy 4 m
Finca la Orden (Guadajira, Badajoz)	38°51'47.8"N 6°39'58.4"W	400	- Drip irrigation in the center of the rows. (3000–3500 m3/ha) - Diameter of the tree canopy 3 m

Figure 2.2. Detail of the Witty® net system used in this study in a fig orchard. A) Witty® net on fig tree; B) Partially dried figs on the bottom net of the Witty® system; C-D) Process of harvesting the dried figs in the Witty® system.

Dried fig samples in fig tree where Witty® system was placed were picked up directly from the net, while in the control trees were harvested following traditional harvesting, picking the dried figs directly by hand from the ground. Two samplings were carried out during the assays, with an interval of 15 days. After harvesting, samples were transported under refrigeration to the laboratory for analysis. The total production of each tree was collected, ranging from 3 to 5 kg for irrigated trees and around 2 kg for rainfed trees. Each tree represented a biological repetition and determinations were carried out in duplicate on each sample.

2.2.- Assessment and quantification of bird and insect damage on dried figs.

To determine the percentage of dried figs damage by birds, all fruit were visually assessed one by one and those with bird pecks, fruit with a break in the longitudinal skin from the stalk to the ostiole or no pulp inside the fruit were classified as damage by birds. After sorting, dried fig with bird damage and undamaged fruits (with no visual defects on the skin) from each sample were weighed, and the results were expressed as a percentage.

To quantify the percentage of fruit with insect damage, 30 fruits were randomly selected from each sample among undamaged dried figs, which are considered eligible for the industry. These fruits were placed individually 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 ±1 ◦C under a 16:8 h L/D photoperiod for 30 days. Fruits were examined every other day, removing fruits with carpophagous larvae or signs of insect presence, such as faeces, webbing, injury or galleries. The larvae detected were observed under a stereomicroscope, checking for the presence of diapausing carpophagous insects, Lepidoptera or Coleoptera. Finally, dried

figs from each category, undamaged or insect damage (Lepidoptera or Coleoptera) were weighed, and the results were expressed as percentage.

2.3.-Physico-chemical parameters

From each sample, 50 undamaged fruits were randomly selected and were used to determine fruit weight, colour and Firmness. Afterwards, they were homogenized using a Mortar Grinder Pulverisette 2 (Fritsch, Germany) for moisture, water activity (a_w), total soluble solids and mycotoxin determinations.

Fruit weight, total soluble solids, firmness and colour were determined as described Galván et al. (2021). In addition, a_w and moisture content were measured using an a_w meter (LabMASTER-aw, Novasina AG, Lachen, Switzerland) and according to AOAC Official Method 934.01, respectively.

2.4.- Microbiological analysis

Approximately 50 g of the sample was aseptically obtained from 10 randomly selected dried figs and diluted 10-fold in peptone water (Condalab, Madrid, Spain) and homogenized. Subsequently, 10-fold serial dilutions were performed in peptone water and 0.1 mL aliquots were inoculated onto agar plates. Mesophilic aerobic bacteria were counted on Plate Count Agar (PCA, Condalab) after incubation at 30ºC for 48 h and fungi (yeast and molds) on acidified potato dextrose agar (PDA, Condalab) after incubation at 25ºC for 4 days. PDA was acidified to pH 3.5 with a sterile 10% (w/v) tartaric acid solution. The results were expressed as log cfu/g.

2.5.- Mycotoxin Extraction and Quantification by Orbitrap Q Exactive Plus Analysis

Mycotoxins were extracted from approximately 5 g of homogenised dried fig samples with acetonitrile/water/acetic acid (79:20:1, v/v/v) at a ratio of 4 mL/g sample following the methodology described by Sulyok et al. (2020). One mL of the extract was diluted with 1 mL of acetonitrile/water/acetic acid (20:79:1, v/v/v) with the aim to enhance chromatographic resolution, filtered through a 0.22 µm pore-size nylon membrane filter and stored in vial at -80ºC until chromatographic analysis.

The chromatographic analysis was common for every mycotoxin determination. Ten microliters of every sample were analysed using a Q Exactive Plus mass spectrometer (Thermo Fischer Scientific) following the methodology described by Cebrián et al., 2020 The flow rate was stablished at 0.4 mL/min, and the mycotoxin analysis was performed with a separation slope for 6 min, 35–98% B, column washing (98% B) for 3 min and 1 min of postconditioning (35% B) resulting in a total run time of 10 min.

Calibration curves from 0.1 to 200 ng/mL were carried out using synthetic commercial standards (Table 2.2). Linearity was estimated in the working range for every mycotoxin by spiking the acetonitrile extraction solution recovered from non-inoculated samples extracted with the methodology described above, in order to subtract the matrix effect. In addition, limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the matrix from untreated samples undergone to the extraction method, and spiked with the mycotoxin standards, as the lowest evaluable concentration level at which the qualifier ion signal exceeds the noise level by a factor of 3.5 and 10, respectively (Table 2.2).

Table 2.2. Mycotoxin standard analyzed, retention time (RT), limit of detection (LOD), limit of quantification (LOQ) and commercial source.

Mycotoxin	**RT (min)**	**LOD (ng/mL)**	**LOQ (ng/mL)**	**Commercial source**
Aflatoxin G1 (AFG1)	1.42±0.02	0.17	0.5	LGC Standard (Middlesex, UK)
Aflatoxin G2 (AFG2)	1.25±0.02	0.17	0.5	LGC Standard
Aflatoxin B1 (AFB1)	1.68±0.02	0.17	0.5	LGC Standard
Aflatoxin B2 (AFB2)	1.49±0.02	0.17	0.5	LGC Standard
Ochratoxin A (OTA)	3.35±0.02	0.33	1	Merck (Darmstadt, Germany)
Ochratoxin B (OTB)	2.47±0.02	0.17	0.5	Merck
Citrinin (CIT)	2.41±0.02	0.17	0.5	Merck
Cyclopiazonic acid (CPA)	4.25±0.02	0.33	1	Merck
Mycophenolic Acid (Mic Ac)	2.34±0.02	0.33	1	Merck
Zearalenone (Zea)	3.39±0.02	0.17	0.5	Merck
Patulin	0.77±0.02	0.33	1	Merck
Penicillic acid	0.93±0.02	0.17	0.5	Merck
O-Methylsterigmatocystin (OM STG)	2.84±0.02	0.17	0.5	Santa Cruz Biotechnology (Heidelberg, Germany)
Fumonisin B1	1.18±0.02	0.66	2	Thermo Scientific Chemicals (Waltham, USA)
Kojic acid	0.66±0.02	0.33	1	MedChemExpress (Monmouth Junction, USA)
Sterigmatocystin (STG)	3.87±0.02	0.17	0.5	Merck
Griseofulvin	2.51±0.02	0.17	0.5	Focus Biomolecules (Plymouth Meeting, USA)
Alternariol (AOH)	3.45±0.02	0.17	0.5	Cayman Chemical (Ann Arbor, USA)
Alternariol-monomethyl ether (AME)	1.85±0.02	0.17	0.5	Cayman Chemical

For mycotoxin determination, the Full MS methodology was utilised with a scan range between 53.4 and 800 *m/z*. For every analysis, an ESI source (HESI II, Fischer Scientific) operating in positive ion mode ESI^+) with spray voltage 4 kV, sheath gas (N_2

> 95%) 30, auxiliary gas (N_2 > 95%) 10, capillary temperature 350 ºC, S-lens RF level 55, auxiliary gas heater temperature 350 ºC.

. Every analysis was carried out with a resolution of 70,000 full widths at half maximum (FWHM). Data analysis and processing were carried out using the FreeStyle™ software, v. 1.5 (Thermo Fisher Scientific). Each mycotoxin was identified by its retention time and its exact mass at ±5 ppm.

2.6.- Statistical analysis

The bird and insect fruit damage percentage, physicochemical and microbiological data were treated with a two-way analysis of variance (ANOVA) using SPSS for Windows, 25.0. Subsequently, the Bonferroni test was applied to compare the mean values obtained and the significance level was set at $p \leq 0.05$. The percentage of samples contaminated with mycotoxin at different levels was determined using Monte Carlo chi-square tests using contingency tables. Statistically significant differences were established by Bonferroni post hoc test. Statistically significant differences were established by the Z-test of two proportion. Finally, principal component analysis (PCA) was performed on the correlation matrix of variables.

3. Results

3.1.- Impact of Witty® system on the level of bird and insect damage on dried figs

Table 2.3 shows the percentage of dried fig damaged by birds and insects obtained by the traditional and Witty® net crop management in the three orchards evaluated. The mean percentage of damaged dried figs by birds ranged from 3.03 to 21.74 (average 11.93) under traditional system and from 0.15 to 2.36 (average 1.02) when Witty® net was used, with significant difference ($p < 0.001$) between orchards and crop management systems. Interestingly, the use of Witty® net minimized bird damage substantially in all orchards evaluated, with the impact being most evident when damage was greatest (p location-system < 0.001).

Table 2.3 Mean percentage ± SD of fruit with damage caused by birds and insects under traditional and Witty® systems at the three orchards evaluated.

System and location	Birds (%)	Insects		
		Total (%)	Lepidoptera (%)	Coleoptera (%)
Arroyomolinos	***5.59 ± 5.74^{b}***	***2.33 ± 4.66b***	***2.00 ± 4.67b***	***0.33 ± 1.15b***
Traditional	11.02 ± 1.27^{1B}	3.33 ± 6.41^{B}	3.33 ± 6.41^{B}	0 ± 0^{B}
Witty®	0.15 ± 0.24	1.33 ± 2.07^{B}	0.67 ± 1.63^{B}	0.67 ± 1.63^{B}
La Nava de Santiago	***1.78 ± 1.58^{c}***	***6.18 ± 6.38^{b}***	***3.44 ± 5.13^{b}***	***1.90 ± 1.99^{b}***
Traditional	3.03 ± 1.11^{C}	8.67 ± 7.98^{B}	5.67 ± 6.51^{B}	1.33 ± 2.07^{B}
Witty®	0.53 ± 0.70	3.69 ± 3.31^{B}	1.22 ± 1.91^{B}	2.46 ± 1.93^{B}
La Orden	***12.05 ± 12.09a***	***20.67 ± 7.2^{a}***	***12.33 ± 9.26^{a}***	***8.33 ± 6.71a***
Traditional	21.74 ± 9.46^{1A}	22.67 ± 5.47^{A}	15.33 ± 9.27^{1}	7.33 ± 8.16^{A}
Witty®	2.36 ± 2.64	18.67 ± 8.64^{A}	9.33 ± 9^{B}	9.33 ± 5.47^{A}
Traditional	***11.93 ± 9.45^{1}***	***11.56 ± 10.49^{1}***	***8.11 ± 8.85^{1}***	***2.89 ± 5.62***
Witty®	***1.02 ± 1.79***	***7.90 ± 9.43***	***3.74 ± 6.50***	***4.15 ± 5.04***
p location	<0.001	<0.001	<0.001	<0.001
p system	<0.001	0.045	0.009	0.200
p location - system	<0.001	0.776	0.681	0.849

[a,b,c] Indicate significant differences $p \leq 0.05$ between the geographic locations.
[A, B, C] Indicate significant differences $p \leq 0.05$ between the geographic locations with the same production system.

[1] Indicate significant differences $p \leq 0.05$ between the production systems in the same geographic location.

Regarding to insect damage on dried figs, the total mean percentage ranged from 3.33 to 22.67 (average 11.56) under traditional system and from 1.33 to 18.67 (average 7.90) when Witty® net was used, showing significant differences between orchards ($p < 0.001$) and crop management systems ($p < 0.05$). However, the data showed that efficiency of the Witty® net depended on the type of insect. The level of Lepidoptera was in general significantly reduced under Witty® net, by contrast the level of Coleoptera was not statistically affected. The latter even showed higher mean values in all orchards under Witty® system.

3.2.- Impact of Witty® system on physicochemical quality

The effect of Witty® net on the physicochemical parameters (fruit weight, moisture, a_w, TSS, firmness and colour) is shown in Table 2.4. Overall, all these parameters were significantly affected by orchard location ($p < 0.001$), whereas the used of Witty® net only statistically influenced fruit weight, firmness, and colour ($p < 0.05$), not significantly affecting the moisture content, a_w and TSS ($p > 0.05$). These three latter parameters showed in both crop management system mean value around 24.5% for moisture content, 0.665 for a_w and 78% for TSS. Regarding to the other three parameters that were influenced by the used of Witty® net, the fruit weight ranged from 7.78 g to 11.48 g. Dried figs under Witty® net presented significantly higher value (10.22 g) compared to traditional crop management (9.74 g). However, this finding was not uniform in all the orchard evaluated (p location-system < 0.001). Specifically, Arroyomolinos and La Orden orchards showed significantly higher value under Witty® net, contrary to La Nava de Santiago orchard, where the dried figs with higher weight were found in traditional crop management. In terms of firmness, the value ranged from 0.52 N to 0.90

N. Dried figs under Witty® net presented significantly lower value (0.67 N) compared to traditional crop management (0.72 N). However, this finding was not extensible to all orchards (p location-system = 0.02). It should be noted that it was strongly influenced by the differences obtained between both crop management systems in the Arroyomolinos orchard ($p < 0.05$). The two other orchards studied showed similar firmness values under traditional and Witty® net crop management, with no significant differences between then ($p > 0.05$). Finally, with respect to the colour parameters, the lightness (L*) varied from 50.6 to 61.19, while chromaticity (C*) ranged from 23.45 to 34.13 and hue angle (h*) from 70.78 to 73.85. Overall, the impact of the use of Witty® net on colour parameters was similar in all orchards. Dried figs under Witty® net crop management presented significantly ($p < 0.001$) higher value of L*, C* and h*, indicating that their skin had a lighter reddish brown colour. Specifically, the mean values of L*, C* and h* of dried figs under Witty® net crop management were 58.93, 31.35 and 73.61, respectively, whereas under traditional system they were 54.04 (L*), 27.16 (C*) and 71.48 (h*).

Table 2.4. Mean percentage ± SD of physicochemical parameters under traditional and Witty® systems at the three orchards evaluated.

System and location	Fuit weight (g)	Moisture content (%)	Water activity (a_w)	Total soluble solids (ºBrix)	Firmness (N)	Colour		
						L*	C*	h*
Arroyomolinos	***11.18 ± 2.03^{b}***	***28.51 ± 3.95^{a}***	***0.71 ± 0.05^{a}***	***74.70 ± 3.19^{b}***	***0.58 ± 0.31^{b}***	***54.70 ± 7.9^{c}***	***26.54 ± 6.04^{c}***	***72.15 ± 3.28^{b}***
Traditional	10.89 ± 1.99^{A}	29.66 ± 5.36^{A}	0.72 ± 0.07^{A}	73.82 ± 4.43^{B}	0.65 ± 0.41^{1B}	50.6 ± 7.57^{C}	23.45 ± 6.35^{C}	70.78 ± 3.44^{B}
Witty®	11.48 ± 2.03^{1A}	27.37 ± 1.54^{A}	0.69 ± 0.02^{A}	75.58 ± 0.97^{B}	0.52 ± 0.16^{C}	58.8 ± 5.84^{1B}	29.62 ± 3.71^{1B}	73.53 ± 2.43^{1}
La Nava de Santiago	***8.00 ± 1.59^{c}***	***22.02 ± 2.86^{b}***	***0.62 ± 0.05^{b}***	***80.70 ± 2.64^{a}***	***0.89 ± 0.43^{a}***	***59.04 ± 6.81^{a}***	***32.28 ± 5.32^{a}***	***72.42 ± 3.71^{b}***
Traditional	8.23 ± 1.77^{1C}	22.67 ± 2.89^{B}	0.62 ± 0.04^{B}	81.03 ± 2.14^{A}	0.90 ± 0.44^{A}	56.89 ± 7.18^{A}	30.42 ± 5.55^{A}	71.40 ± 4.01^{B}
Witty®	7.78 ± 1.35^{B}	21.36 ± 2.94^{B}	0.62 ± 0.06^{B}	80.38 ± 3.24^{A}	0.89 ± 0.41^{A}	61.19 ± 5.68^{1A}	34.13 ± 4.37^{1A}	73.44 ± 3.07^{1}
La Orden	***10.75 ± 2.29^{a}***	***22.93 ± 1.18^{b}***	***0.67 ± 0.04^{a}***	***78.75 ± 2.88^{a}***	***0.61 ± 0.27^{b}***	***55.73 ± 6.48^{b}***	***28.94 ± 4.66^{b}***	***73.05 ± 3.09^{a}***
Traditional	10.10 ± 2.11^{B}	22.34 ± 0.85^{B}	0.67 ± 0.03^{AB}	78.55 ± 1.77^{A}	0.61 ± 0.28^{B}	54.64 ± 6.43^{B}	27.59 ± 4.54^{B}	72.25 ± 3.2^{A}
Witty®	11.39 ± 2.28^{1A}	23.52 ± 1.23^{B}	0.67 ± 0.05^{A}	78.95 ± 3.87^{A}	0.61 ± 0.25^{B}	56.81 ± 6.35^{1C}	30.29 ± 4.41^{B}	73.85 ± 2.76^{1}
Traditional	***9.74 ± 2.26***	***24.89 ± 4.82***	***0.67 ± 0.06***	***77.80 ± 4.19***	***0.72 ± 0.4^{1}***	***54.04 ± 7.53***	***27.16 ± 6.22***	***71.48 ± 3.61***
Witty®	***10.22 ± 2.59^{1}***	***24.08 ± 3.19***	***0.66 ± 0.05***	***78.30 ± 3.47***	***0.67 ± 0.33***	***58.93 ± 6.22^{1}***	***31.35 ± 4.62^{1}***	***73.61 ± 2.77^{1}***
p location	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p system	<0.001	0.293	0.469	0.477	0.043	<0.001	<0.001	<0.001
p location - system	<0.001	0.169	0.465	0.377	0.02	<0.001	<0.001	0.019

[a,b,c] Indicate significant differences $p \leq 0.05$ between the geographic locations.

[A, B, C] Indicate significant differences $p \leq 0.05$ between the geographic locations with the same production system.

[1] Indicate significant differences $p \leq 0.05$ between the production systems in the same geographic location.

3.3.- Impact of Witty® system on microbiological quality and mycotoxin accumulation.

Microbial counts, yeasts, moulds and mesophilic bacteria, of dried figs from traditional and Witty® net crop management at the three orchards evaluated are shown in Figure 2.3.

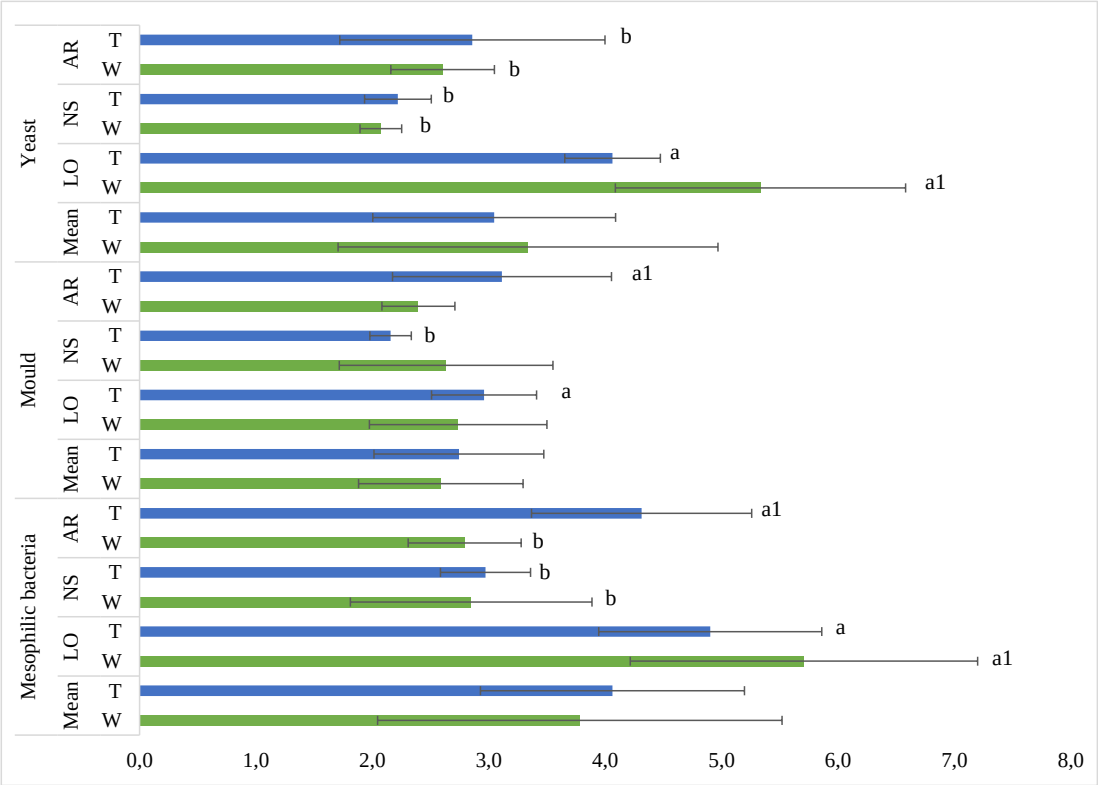


Figure 2.3 Mean counts ± SD (log cfu/g) of yeasts, moulds and mesophilic bacteria in dried figs under traditional (T) and Witty® (W) crop management at the three orchards evaluated (Arroyomolinos: AR; La Nava de Santiago: NS; La orden: LO). [a,b,c] Indicate significant differences $p \leq 0.05$ between geographic locations with the same production system. [1] Indicate significant differences $p \leq 0.05$ between production systems in the same geographic location.

Mesophilic bacteria count ranged from 2.8 to 5.7 log cfu/g, with mean counts for traditional and Witty® net systems of 4.1 and 3.8 log cfu/g, respectively. The count of fungi was in general lower, with yeast and mold counts ranged from 2.1 to 5.3 log cfu/g and from 2.2 to 3.1 log cfu/g, respectively. Regarding to the mean count of these latter microbial groups by crop management system, yeast varied from 3 log cfu/g under traditional system to 3.3 log cfu/g under Witty® net, whereas moulds varied from 2.7 log cfu/g under traditional system to 2.6 log cfu/g under Witty® net. Overall, the data showed

significant differences ($p < 0.05$) among orchard location for each microbial group, however, the crop management system did not statistically ($p < 0.05$) influence the counts of the three microbial groups studied.

On the other hand, the presence of moulds may lead to mycotoxin contamination in dried figs. Table 2.5 shows the percentage of samples that contained a specific mycotoxin at different ranges by orchard location and crop management system. In total 13 different mycotoxins were detected, whose amount was mainly influenced by the orchard location, finding significant differences ($p < 0.05$) in 11 of the 13 mycotoxins detected between the orchards evaluated. By type of mycotoxin, aflatoxins were found above the LOD in a higher percentage of samples in the Arroyomolinos orchard, exceeding the 8 µg/kg limit for AFB1, AFB2, AFG1 and AFG2 in 10.4%, 4.17%, 14.58% and 6.25%, respectively, while in the other two orchards evaluated, only AFB2 was found in 12.5% of the samples in La Orden orchard at levels ranging from LOD to 8 µg/kg. In addition, O-methylsterigmatocystin (OM-STG), which is a metabolic intermediate of aflatoxins, was also detected in higher level in Arroyomolinos orchard, with 10.42% of samples above the LOD. By contrast, ochratoxin A (OTA) y B (OTB) were no detected in Arroyomolinos orchard, whereas in La Nava de Santiago and La Orden orchards were found at significant higher levels ($p < 0.05$). OTA was found at level above 8 µg/kg in La Nava de Santiago and La Orden orchards in 31.94% and 15.97% of the samples, respectively, while OTB at this level was detected in a lower percentage of samples, 8.33% in both orchards. Other mycotoxins detected at different levels were citrinin (CIT), mycophenolic acid (Mic Ac), kojic acid (Kojic Ac), zearalenone (ZEA), alternariol (AOH) and alternariol-monomethyl ether (AME). CIT and Mic Ac were only found in 2.08% of the samples at lower level (LOD-8 µg/kg) in Arroyomolinos and La Nava de Santiago orchards, respectively, without significant ($p > 0.05$) differences between

orchards. Similarity, kojic Ac. presented a low occurrence, being only found in 8.33% and 6.25% of the samples from La Nava de Santiago and La Orden orchards, respectively, at range >40 µg/kg. Finally, the occurrence of ZEA, AOH and AME were significantly higher ($p < 0.05$) in La Orden orchard with 60.42%, 33.33% and 16.58% of the samples above the LOD, respectively. It should be noted that the percentage of samples of these three latter mycotoxins in La Orden orchard at high level was also noticeable. AME and AOH were found in 8.33% and 16.67% of the samples at range >40 µg/kg and ZEA in 29,17% at range 8-40 µg/kg.

Table 2.5. Percentage of samples of dried figs contaminated with mycotoxins at different levels under traditional (T) and Witty® (W) systems at the three orchards evaluated.

Mycotoxins		Arroyomolinos			La Nava de Santiago			La orden			TOTAL	
		Total	T	W	***Total***	T	W	***Total***	T	W	T	W
AFB1	<LOD	***83.33^{b}***	83.33^{B}	83.33^{B}	***100^{a}***	100^{A}	100^{A}	***100^{a}***	100^{A}	100^{A}	94.44	94.44
	LOD - 8 µg/kg	***6.25^{a}***	-^{B}	12.5^{A1}	***-^{b}***	-^{B}	-^{B}	***-^{b}***	-^{B}	-^{B}	-	4.17^{1}
	8 - 40 µg/kg	***4.17^{a}***	4.17	4.17	***-^{b}***	-	-	***-^{b}***	-	-	1.39	1.39
	>40	***6.25^{a}***	12.5^{A1}	-^{B}	***-^{b}***	-^{B}	-^{B}	***-^{b}***	-^{B}	-^{B}	4.17^{1}	-
AFB2	<LOD	***87.5^{b}***	83.33^{B}	91.67^{AB}	***100^{a}***	100^{A}	100^{A}	***87.5^{b}***	83.33^{B}	91.67^{AB}	88.89	94.44^{1}
	LOD - 8 µg/kg	***8.33^{a}***	8.33^{AB}	8.33^{AB}	***-^{b}***	-^{B}	-^{B}	***12.5^{a}***	16.67^{A}	8.33^{AB}	8.33	5.56
	8 - 40 µg/kg	***4.17^{a}***	8.33^{1}	-	***-^{b}***	-	-	***-^{b}***	-	-	2.78^{1}	-
	>40	***-***	-	-	***-***	-	-	***-***	-	-	-	-
AFG1	<LOD	***83.33^{b}***	83.33^{B}	83.33^{B}	***100^{a}***	100^{A}	100^{A}	***100^{a}***	100^{A}	100^{A}	94.44	94.44
	LOD - 8 µg/kg	***2.08***	-	4.17	***-***	-	-	***-***	-	-	-	1.39
	8 - 40 µg/kg	***6.25^{a}***	-^{B}	12.50^{A1}	***-^{b}***	-^{B}	-^{B}	***-^{b}***	-^{B}	-^{B}	-	4.17^{1}
	>40	***8.33^{a}***	16.67^{A1}	-^{B}	***-^{b}***	-^{B}	-^{B}	***-^{b}***	-^{B}	-^{B}	5.56^{1}	-
AFG2	<LOD	***89.58^{b}***	83.33^{B}	95.83^{AB1}	***100^{a}***	100^{A}	100^{A}	***100^{a}***	100^{A}	100^{A}	94.44	98.61^{1}
	LOD - 8 µg/kg	***4.17^{a}***	4.17	4.17	***-^{b}***	-	-	***-^{b}***	-	-	1.39	1.39
	8 - 40 µg/kg	***4.17^{a}***	8.33^{1}	-	***-^{b}***	-	-	***-^{b}***	-	-	2.78^{1}	-
	>40	***2.08***	4.17	-	***-***	-	-	***-***	-	-	1.39	-
OM STG	<LOD	***89.58^{b}***	91.67^{AB}	87.5^{B}	***100^{a}***	100^{A}	100^{A}	***100^{a}***	100^{A}	100^{A}	97.22	95.83
	LOD - 8 µg/kg	***10.42^{a}***	8.33^{AB}	12.50^{A}	***-^{b}***	-^{B}	-^{B}	***-^{b}***	-^{B}	-^{B}	2.78	4.17
	8 - 40 µg/kg	***-***	-	-	***-***	-	-	***-***	-	-	-	-
	>40	***-***	-	-	***-***	-	-	***-***	-	-	-	-

OTA	<LOD	***100^{a}***	100^{A}	100^{A}	***58.33^{c}***	50^{C}	66.67^{BC1}	***81.25^{b}***	83.33^{B}	79.17^{B}	77.78	81.94
	LOD > 8 µg/kg	**-**	-	-	**-**	-	-	**-**	-	-	-	-
	8 - 40 µg/kg	***-^{c}***	-^{B}	-^{B}	***4.86^{a}***	8.33^{AB}	20.83^{A1}	***1.39^{b}***	-^{B}	8.33^{AB1}	2.78	9.72^{1}
	> 40	***-^{c}***	-^{C}	-^{C}	***27.08^{a}***	41.67^{A1}	12.5^{B}	***14.58^{b}***	16.67^{B}	12.5^{B}	19.44^{1}	8.33
OTB	<LOD	***100^{a}***	100^{A}	100^{A}	***68.75^{c}***	62.5^{C}	75^{BC}	***85.42^{b}***	87.5^{B}	83.33^{BC}	83.33	86.11
	LOD > 8 µg/kg	***-^{c}***	-^{B}	-^{B}	***22.92^{a}***	20.83^{A}	25^{A}	***6.25^{b}***	4.17^{B}	8.33^{AB}	8.33	11.11
	8 - 40 µg/kg	***-^{b}***	-^{B}	-^{B}	***8.33^{a}***	16.67^{A1}	-^{B}	***6.25^{a}***	4.17^{AB}	8.33^{AB}	6.94^{1}	2.78
	> 40	**-**	-	-	**-**	-	-	***2.08***	4.17	-	1.39	-
CIT	<LOD	***97.92***	95.83	100	***100***	100	100	***100***	100	100	98.61	100.00
	LOD > 8 µg/kg	***2.08***	4.17	-	**-**	-	-	**-**	-	-	1.39	-
	8 - 40 µg/kg	**-**	-	-	**-**	-	-	**-**	-	-	-	-
	> 40	**-**	-	-	**-**	-	-	**-**	-	-	-	-
Mic Ac	<LOD	***100***	100	100	***97.92***	95.83	100	***100***	100	100	98.61	100.00
	LOD - 8 µg/kg	**-**	-	-	***2.08***	4.17	-	**-**	-	-	1.39	-
	8 - 40 µg/kg	**-**	-	-	**-**	-	-	**-**	-	-	-	-
	>40	**-**	-	-	**-**	-	-	**-**	-	-	-	-
ZEA	<LOD	***87.5^{a}***	87.5^{A}	87.5^{A}	***83.33^{a}***	79.17^{A}	87.5^{A}	***39.58^{b}***	50^{B1}	29.17^{B}	72.22	68.06
	LOD - 8 µg/kg	***12.50^{b}***	12.50^{B}	12.5^{B}	***14.58^{b}***	16.67^{B}	12.50^{B}	***31.25^{a}***	20.83^{AB}	41.67^{A1}	16.67	22.22
	8 - 40 µg/kg	***-^{b}***	-^{B}	-^{B}	***2.08^{b}***	4.17^{B}	-^{B}	***29.17^{a}***	29.17^{A}	29.17^{A}	11.11	9.72
	>40	**-**	-	-	**-**	-	-	**-**	-	-	-	-
Kojic Ac	<LOD	***100^{a}***	100^{A}	100^{A}	***91.67^{b}***	83.33^{B}	100^{A1}	***93.75^{b}***	91.67^{AB}	95.83^{AB}	91.67	98.61^{1}
	LOD - 8 µg/kg	**-**	-	-	**-**	-	-	**-**	-	-	-	-
	8 - 40 µg/kg	**-**	-	-	**-**	-	-	**-**	-	-	-	-
	>40	***-^{b}***	-^{B}	-^{B}	***8.33^{a}***	16.67^{A1}	-^{B}	***6.25^{a}***	8.33^{AB}	4.17^{AB}	8.33^{1}	1.39

AME	<LOD	***100^{a}***	100^{A}	100^{A}	***100^{a}***	100^{A}	100^{A}	***85.42^{b}***	87.50^{B}	83.33^{B}	95.83	94.44
	LOD - 8 µg/kg	***-^{b}***	-	-	***-^{b}***	-	-	***4.17^{a}***	8.33^{1}	-	2.78^{1}	-
	8 - 40 µg/kg	***-***	-	-	***-***	-	-	***2.08***	4.17	-	1.39	-
	>40	***-^{b}***	$-^{B}$	$-^{B}$	***-^{b}***	$-^{B}$	$-^{B}$	***8.33^{a}***	$-^{B}$	16.67^{A1}	-	5.56^{1}
AOH	<LOD	***91.67^{a}***	83.33^{B}	100^{A1}	***89.58^{a}***	79.17^{B}	100^{A1}	***66.67^{b}***	66.67^{B}	66.67^{B}	76.39	88.89^{1}
	LOD - 8 µg/kg	***6.25***	12.5^{A1}	$-^{B}$	***6.25***	12.5^{A1}	$-^{B}$	***8.33***	$-^{B}$	16.67^{A1}	8.33	5.56
	8 - 40 µg/kg	***2.08***	4.17^{AB}	$-^{B}$	***4.17***	8.33^{AB1}	$-^{B}$	***8.33***	16.67^{A1}	$-^{B}$	9.72^{1}	-
	>40	***-^{b}***	$-^{B}$	$-^{B}$	***-^{b}***	$-^{B}$	$-^{B}$	***16.67^{a}***	16.67^{A}	16.67^{A}	5.56	5.56

Regarding to the impact of the crop management system, it had a lesser impact than orchard location in mycotoxin occurrence (Table 2.5). However, overall, notably the use Witty® net resulted in a decrease in average mycotoxin levels in dried fig samples compared to the traditional system. Specifically, dried fig samples obtained from trees under Witty® net system contained significant ($p < 0.05$) higher percentage of samples below the LOD for AFB2, AFG2, kojic Ac and AOH. In addition, this system also led to a statically ($p < 0.05$) lower percentage of dried fig samples contaminated at level ranging from 8 to 40 µg/kg for OTB, AFG2 and AOH and at level >40 µg/kg for OTA, AFB1, AFG1 and kojic Ac. By contrast, for the other three mycotoxins detected, CIT, Mic Ac and ZEA, no significant differences ($p < 0.05$) in the percentage of samples contaminated were found between both crop management system.

3.4.- Multivariate analysis.

Figure 2.4 and 2.5 shows the PCA loading and score plots of the studied parameters according to orchard location and different crop management applied. The result of the factor analysis showed a clear impact of orchard location on the studied parameters along the first (PC1) and second (PC2) components, which explained the 42.5% and 30.5% of the variance, respectively (Fig. 2.4A and ·B). Dried fig samples from La Orden orchard were located on the negative score of the PC1 and positive score of the PC2, which were linked to damage caused by insects, Lepidoptera and Coleoptera, yeast and mesophilic bacteria counts and mycotoxin contamination by ochratoxins (OTA and OTB), ZEA, kojic Ac. and *Alternaria* toxins (AME and AOH). On the contrary side of the PC2 were located dried fig samples from La Nava de Santiago Orchard, which were associated with physic-chemical parameters TSS, colour (L* and C*) and firmness and Mic Ac mycotoxin. Finally, samples from Arroyomolinos orchard were located on the right side of the PC1 axis closed to PC2 axis, being mainly associated with the physic-

chemical parameters content of moisture and a_w and mycotoxin occurrence of aflatoxins (AFB1, AFB2, AFG1 and AFG2), OM STG and CIT. It can also be seen that mould counts, bird damage and fruit weight were concentrated on the positive axis of PC1 and PC2 attributed to their positive correlation with samples from La Orden and Arroyomolinos orchards.

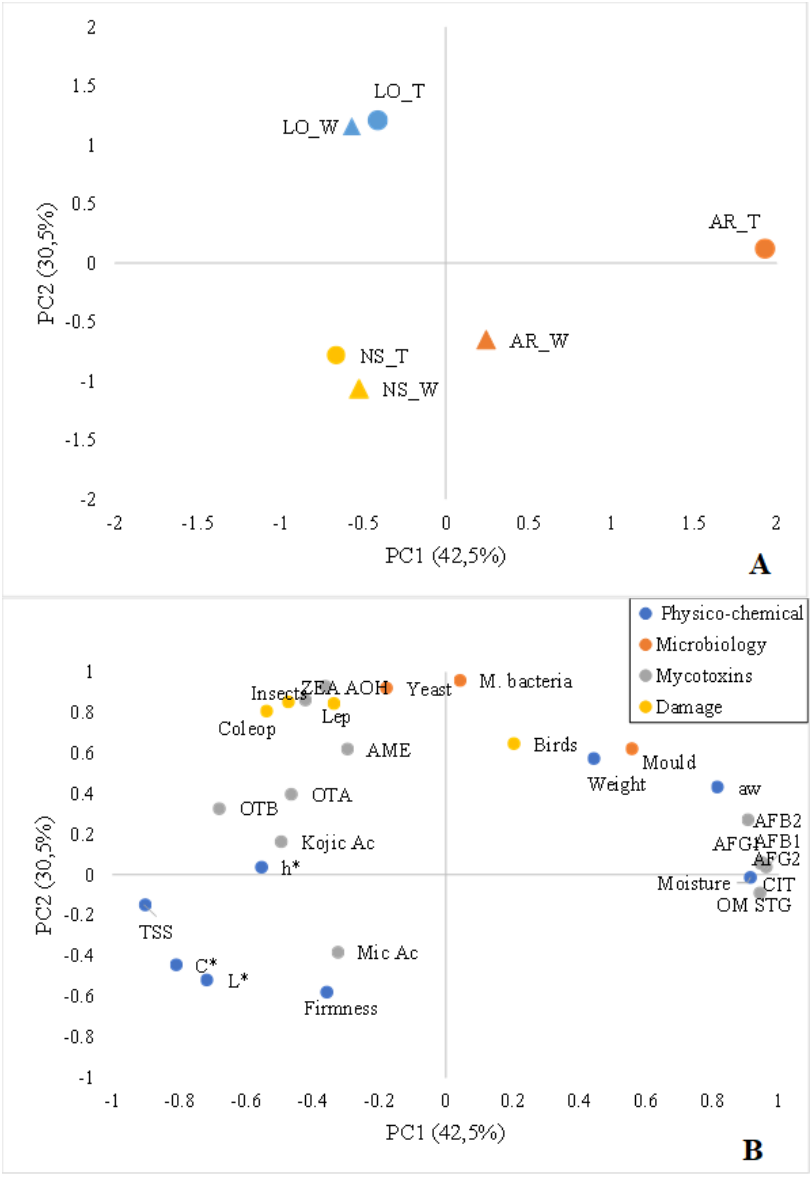


Figure 2.4. Projection of samples grouped according to orchard location (Arroyomolinos AR: orange colour; La Nava de Santiago NS: yellow colour; La orden LO: blue colour) and crop management (Traditional: circle; Witty® net: triangle) in the space defined by the first two components (PC1/PC2; Fig. 2.4A and Fig 2.4B). Physico-chemical parameters: blue circle (Moisture; water activity: aw; Colour: L*, C* and h*; Total soluble solid: TSS; Firmness; Fruit weight: weight); Microbiology: orange circle (Mesophilic bacteria: M bacteria; Yeast; Moulds); Mycotoxins: grey circle (Aflatoxins: AFB1, AFB2, AG1 and AG2; O-Methylsterigmatocystin: OM STG; Ochratoxins: OTA and OTB; Micophenolic acid: Mic Ac; Kojic acid. Kojic Ac.; Zearalenone: ZEA; Citrinin: CIT; Alternariol-monomethyl ether: AME; Alternariol: AOH); Damage: yellow circle (Bird; Insects; Coleoptera: Coleop; Lepidoptera: Lep).

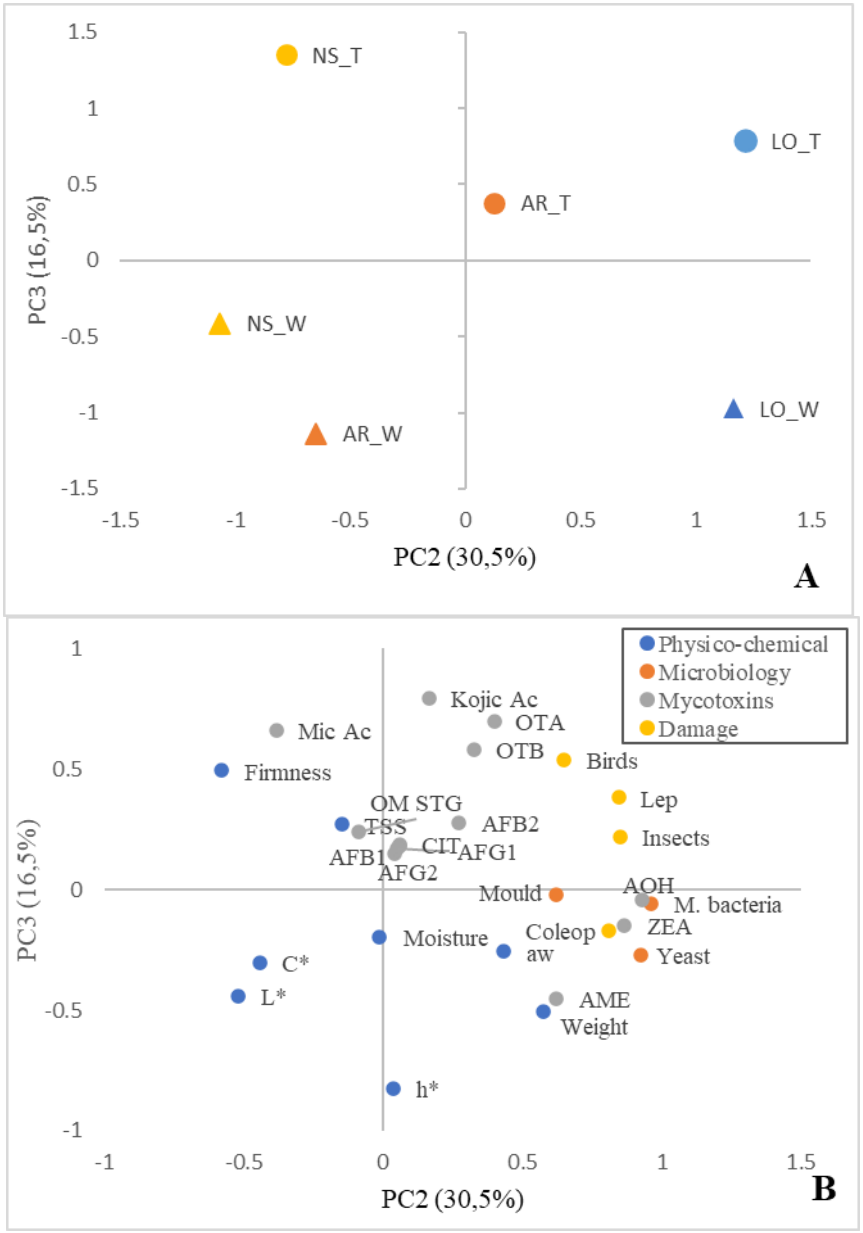


Figure 2.5. Projection of samples grouped according to orchard location (Arroyomolinos AR: orange colour; La Nava de Santiago NS: yellow colour; La orden LO: blue colour) and crop management (Traditional: circle; Witty® net: triangle) in the space defined by the the second and third components (PC2/PC3; Figure 2.5A and Figure 2.5B). Physico-chemical parameters: blue circle (Moisture; water activity: a_w; Colour: L*, C* and h*; Total soluble solid: TSS; Firmness; Fruit weight: weight); Microbiology: orange circle (Mesophilic bacteria: M bacteria; Yeast; Moulds); Mycotoxins: grey circle (Aflatoxins: AFB1, AFB2, AG1 and AG2; O-Methylsterigmatocystin: OM STG; Ochratoxins: OTA and OTB; Micophenolic acid: Mic Ac; Kojic acid. Kojic Ac.; Zearalenone: ZEA; Citrinin: CIT; Alternariol-monomethyl ether: AME; Alternariol: AOH); Damage: yellow circle (Bird; Insects; Coleoptera: Coleop; Lepidoptera: Lep).

On the other hand, the results of the factorial analysis according to crop management system (traditional and Witty® net) were mainly explained by the third component (PC3), which explained the 16.5% of the variability (Figure 2.5A and B).

Interestingly, dried fig samples from the traditional system were located on the positive axis of PC3 and clearly separated from sample of Witty® net system, which were in negative axis of PC3. This distribution can be attributed to the fact that samples from traditional system presented more damage caused by birds and insects (Lepidoptera), greater firmness and content of TSS due to lower moisture and a_w, and more occurrence of most of the mycotoxins detected, mainly ochratoxins, aflatoxins, OM STG, Mic Ac, CIT and kojic Ac. By contrast, samples from Witty® net system were mainly positive correlated with higher value of the colour parameters (L*, C* and h*), moisture, a_w, fruit weight and occurrence of AME mycotoxin.

4. Discussion

Traditional production system for dried fig is the most common in the Mediterranean area, which involve low-density orchards characterised by very limited management and irrigation and fruit harvesting from the ground once a week (Flaishman et al., 2008; Lopéz-Corrales et al., 2012). In the last decade there has been an increase in demand and, consequently, in production due to the scientific evidence of its nutritional and pharmaceutical benefits (Arvaniti et al., 2019; FAOSTAT, 2021). In this context, to enhance profitability and dried fig quality, new crop management strategies are introduced such as soil management, irrigation and fertilization control, mesh netting system for harvesting, high-density formation using dwarfing rootstock and training systems that ensure maximum sunlight interception, as well as better adaptation of these systems to facilitate operations such as harvesting, pruning, and others (Iglesias, 2019; Galván et al., 2021; Jafari et al., 2022). Nevertheless, dried fig during its ripening and drying is highly susceptible to different pest, mainly birds, insects and moulds, which cause important yield and quality losses, even sanitary problem by the occurrence of mycotoxins (Chapter 1). These issues highlight the importance of implementing crop management strategies to improve the yield of high quality and safety dried figs. As far as we know, the influence of net covering the tree canopy, such as Witty® net system, during harvesting period on dried fig quality fruit are chiefly unknown. In this study, we evaluate the impact of Witty® net system in comparison with traditional harvesting management on pest control, physico-chemical and microbiology quality and mycotoxin occurrence.

Birds have been recently reported as the main cause of dried fig damage, being the responsible of important yield losses that can vary from 1.13% to 33.39%, depending on the crop management level (Galván et al., 2021; Chapter 1). Overall, the control of

birds on fig crop is not easier. So, the use of a good bird-repellent system on fig crop is crucial to increase its production. The most common bird repellents in Extremadura production area are different sound scaring and visual bird deterrents, showing the best results on fruit crop when both are combined. However, the birds end up adapting to them, which reduces their effectiveness. (Bishop et al., 2003; Tracey et al., 2007; Simon, 2008). As a more promising alternative methodology, it is the use of a physical barrier such as nets that completely cover the tree (Simon, 2008, Singh et al., 2022). However, in Extremadura, this method is difficult to apply due to the large canopy of the trees and its high cost. In this regard, Witty® net has been designed to be used in fig trees with different canopy sizes (Figure 2.2). Our results confirmed its effectiveness of this system on different fig tree orchards, reducing sharply bird damage average from around 12% to 1%. After birds, insects have been reported to cause losses around 5% on dried figs from Extremadura production area (Chapter 1). Likewise, to bird damage, the use of Witty® net reduced significantly the insect damage from 11.56% to 7.90%, attributed to the decrease of Lepidoptera. However, in general the effectiveness on insect control was lower than birds, which may be explained due to insects can infest figs before place Witty® net or be trapped under it. In addition, their smaller size may allow them to find gaps through which to overcome the netting and infest the figs. It is worth mentioning that between insect types, Lepidoptera and Coleoptera, we observed significantly differences in their control. We have reported in our previous work the main insect species in dried fig from Extremadura production area. Among Lepidoptera, highlighted the presence of *Cadra figulilella*, *Cadra abstersella* and *Ectomyelois ceratoniae*, while the predominant Coleptera was *Carpophilus hemipterus* (Chapter 1). The size of the adult of main species of Lepidoptera identified in dried fig from Extremadura are bigger than Coleptera, which are around 3-4 mm and may easily overcome the Witty® net (Ben-Yakir

and Costa, 2022). In this context, better reduction on insect damage may be obtained by combination Witty® net with biological control strategies such as pheromones or predatory insects (Sigh et al., 2022; Reis et al., 2023).

In addition to reducing the pest damage on dried figs, an essential factor is that Witty® net must not negatively affect the general dried fig quality after harvesting. We showed that the physico-chemical parameters moisture content, a_w and TSS were not influenced by using Witty® net. By contrast, its use led to fruit with greater size, lower firmness and lighter reddish brown colour. These modifications may be attributed to the lower radiation to which fig trees and fruits are exposed under Witty® net. This net provides between 15 and 18% shade to the tree. It is well known that the temperature and radiation intervene in the physiology of the crop and fruit development affecting the postharvest fruit quality at a variable level depending on the fruit and experimental conditions (Tombesi et al., 1993; Beckles et al., 2012, Rivera et al., 2017; Cucunubo Bosa et al., 2019; Byeon and Lee, 2020; Jokar et al., 2021). Cucunubo Bosa et al. (2019) reported in strawberries that the modification of radiation has a strong influence on postharvest fruit quality, obtaining higher fruit weight under favorable conditions. In figs, Beyeon and Lee (2020) compared fig postharvest quality obtained from two production systems (open field vs. greenhouse), demonstrating that different microclimatic conditions affect fruit size and weight loss during postharvest storage. Similarly, Jokar et al. (2021) in 'Sabz' and 'Siah' fig cultivar in Iran production area showed that the use of yellow and blue photo-selective net during the whole ripening period of the fruit (3 months) influence the tree physiology and fruit quality at a different rate. Contrary to our work, they found lower fig size under both net systems, although the experimental conditions, net type and fig variety differ to our work. So, the various responses on fruit size reported in previous research suggest the type of net and installation period have to

be optimized for higher quality fruit production. On the other hand, the larger size of dried figs harvested with Witty® net system may be responsible for their lower firmness, since there were no differences in the moisture content and a_w. This result is in line with the findings of Galván et al. (2021) in the same variety, who also reported the lowest firmness in bigger dried figs. Respect to the colour, the typical brown colour of dried figs is consequently that during drying process phenolic compounds are used by enzyme such as polyphenol oxidase, resulting in oxidized forms of them, which subsequently polymerize into brown compounds (Perera, 2005). Moreover, in the formation of this colour also intervene non-enzymatic such as the Maillard reaction; the high sugar content of figs and high temperatures favor this reaction between reducing sugar and amino acids, as well as caramelization and ascorbic acid browning (Perera, 2005; Yemiş et al., 2012). As expected, the protection provided by Witty® net against sunlight and radiation may limit these reactions (Echavarría et al., 2012; Galván et al., 2023). Therefore, the use of Witty® net influence physico-chemical parameters that play a decisive role in the final sensory quality and thereby determine consumer acceptance (Polat and Siddiq, 2012; Ansari et al., 2014). Notably, these modifications can be considered positive on the global quality. Greater weight of the dried figs can lead to better commercial clase according to Standard DDP-14 (UNECE, 2016). Moreover, softer firmness and lighter reddish brown colour are highly valuable sensory characteristics for consumer acceptance (Rahemi and Jafari, 2005; Mat Desa et al., 2019; Villalobos et al., 2019).

On the other hand, dried figs are susceptible to be contaminated with a wide range of toxigenic moulds during their ripening process due to their nutritional and physicochemical traits, which may compromise their quality and mycotoxin occurrence (Sulyok et al., 2020; Galván et al., 2023). To date, the Commission Regulation (EC) has set only maximum legal limits in dried figs for aflatoxins, 6 µg/kg for AFB1 and 10 µg/kg

for total aflatoxins (EC Nº. 1058/2012) and for ocrhatoxin A 8 µg/kg (EC Nº2022/1370). However, a wide range of mycotoxins have been found in dried figs, which remain unregulated (Di Sanzo et al., 2018; Sulyok et al., 2020). Witty® net system did not influence moulds counts. However, mycotoxin occurrence was clearly affected by orchard location, and notably also by Witty® net, which significantly reduced the level of most of the mycotoxins detected. Previous works have reported that environmental or climate conditions as temperature and humidity strongly influence mould colonization and mycotoxin production in fruit (García-Cela et al., 2014; Medina et al., 2015ab; Galván et al., 2023). In traditional system the fig begins to dry on the tree, until it falls to the ground, where it completes its drying process until are harvesting by hand, by contrast the use of Witty® net avoid the contact of the dried figs with the ground, makes harvesting easier, and represents a physical barrier against birds and insects. So, dried figs under traditional system are more exposure to airbone mould spores, insects and birds that can spread spore from toxigenic mould and contaminate figs (Paster and Barkai-Golan, 2008; Mostowfizadeh-Ghalamfarsa et al., 2022) and to the specific soil mycobiota of each orchard, which is a relevant source of mould contamination (Özer et al., 2012; Galván et al., 2023).

5. Conclusion

This is the first known report on the application of a net, commercially denominated Witty® system, which completely covers the fig tree canopy during the harvesting dried fig period. Our results show that the use of Witty® system led to a considerable reduction in the percentage of damaged fruit caused by birds and Lepidoptera insects. It is worth noting that the efficiency in the control of the main cause of damage in dry figs, birds, was notably, limiting it to approximately 1 %. In addition, its use modified positively physicochemical parameters that play a decisive role in sensory quality. Dried figs obtained with the Witty® system compared to the traditional system had significant greater fruit weight, softer firmness and lighter reddish brown colour. In contrast, the use of Witty® system did not influence the microbial counts of bacteria, yeasts and moulds, However, outstanding, its use significantly reduced the occurrence of most of the mycotoxins detected. Therefore, although the installation of Witty® net involves an initial cost for farmers, our findings demonstrate its effectiveness in reducing pest incidences, providing dried fig with better physicochemical and hygienic-sanitary quality. These scientific findings, together with the improvement that the Witty® net brings to harvesting operations, suggest that its use could lead to more profitable production and also positively influence the marketability of dried figs.

CHAPTER 3

CHAPTER 3

Freezing treatments as an alternative to conventional pest control in dried figs and their effect on global fruit quality

1. Abstract

One of the main problems for the dried fig industry is pest control during storage and subsequent marketability. For this reason, the dried fig industry has traditionally applied pest control treatments with chemical-based insecticides, usually phosphine. However, current consumers want healthier products and a reduction in chemical use to reduce the environmental impact, so the dried fig industry is seeking alternatives to chemical treatments. Freezing has been shown to be an effective alternative for controlling dried fig pests. Treatments of 1 day at −18°C, 2 days at −10°C, and 7 days at −5°C reduced pest incidence, with up to 100% efficiency, during the subsequent storage of dried figs. Moreover, these freezing treatments provided better quality dried figs, both from a sensory point of view, as was the case of dried figs subjected to 7 days at −5°C, and from a bioactive compound point of view. Dried figs exposed for 2 days at −10°C maintained a higher concentration of phenolic compounds. We conclude that freezing would be an appropriate alternative to phosphine treatments for pest control in dried figs.

Keywords: Dried figs, Pest control, Freezing, Insects, Fruit quality, Treatment.

2. Materials and methods

The experimental designs 1 and 2 are shown graphically in the Figure 3.1.

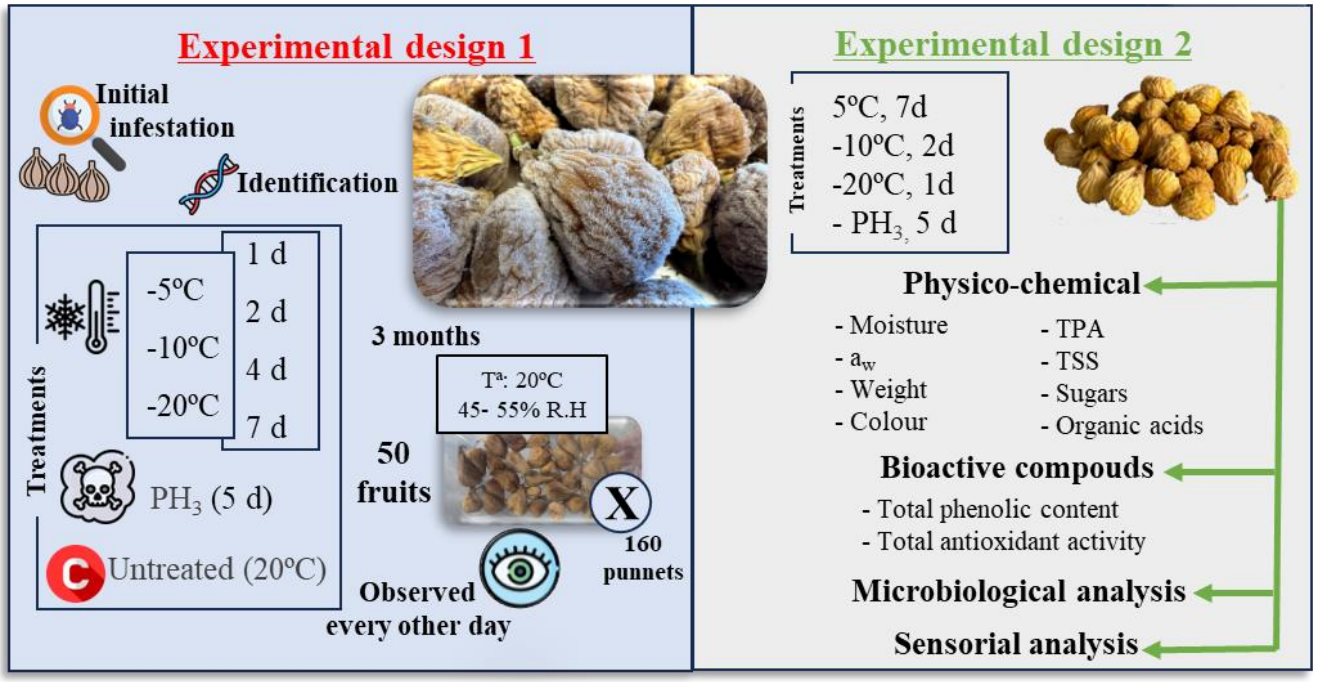


Figure 3.1. Experimental design 1 y 2.

2.1 Experimental design 1: Use of different freezing temperatures and times to control dried fig storage insect pests

2.1.1. Plant material

The experiment was carried out with sun-dried figs of the Calabacita variety with a high percentage of natural insect infestation provided by the Frutas Villacruz S.L. company (Villar de Rena, Badajoz, Spain; 39° 4′ 36″ N; 5° 48′ 42″ W) during the 2020 season.

The initial infestation of dried figs was estimated by dissecting 30 fruits (5 replicates) and observing them under a stereomicroscope. Figs were classified into fruit harbouring carpophages of the order Lepidoptera or Coleoptera, and 20 individuals were randomly selected from each to determine the species. To identify the insects at the

species level by DNA barcoding, the genomic DNA from each individual insect was extracted using the NucleoSpin DNA Insect Mini Kit (Machery-Nagel, Düren, Germany). The region of the mitochondrial cytochrome oxidase subunit I gene was amplified with the universal primers LCO1490 and HCO2198 (Folmer et al., 1994) using PCR reagents and the conditions described in Chapter 1. The PCR products obtained were purified using a GeneJET PCR Purification Kit (Thermo Fisher Scientific, Waltham, MA) and sequenced using 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 identification of the insects was determined based on the highest score.

After insect characterisation, about 5 kg of randomly selected dried figs were placed in plastic fruit boxes for each treatment (n = 12) and stored in different freezing rooms under different temperatures and time conditions. Freezing was performed at 3 temperatures, −5, −10 and −18°C (relative humidity (RH), 50–70%), and four time periods, 1, 2, 4 and 7 days. Temperature and relative humidity were recorded throughout the different treatments using a datalogger in each freezing room (EasyLog EL-USB-2-LCD, Lascar Electronics, Wiltshire, UK); data are shown in Figure 3.2. Additionally, a 5 kg batch was treated with aluminium phosphide (Phostoxin®, DETIA DEGESCH GmbH, Laudenbach Germany) for 5 days in an airtight container, according to the current industrial process, and used as a negative control. Finally, untreated dried figs were placed in a cold room at 20°C as a positive control. One positive control for each freezing temperature was performed (3 positive controls). Subsequently, 300 dried figs for each treatment were placed in 10 transparent polypropylene punnets (26×16×8 cm) containing 30 dried figs each. The upper part was covered with fine mesh to allow ventilation while preventing insects from escaping. In total, there were 160 punnets (120 from freezing

treatments, 30 from dried fig untreated and 10 from phosphine treatment). All samples were stored in a cold room at 20°C and 45–55% RH for 90 days, and insect presence was visually inspected every other day.

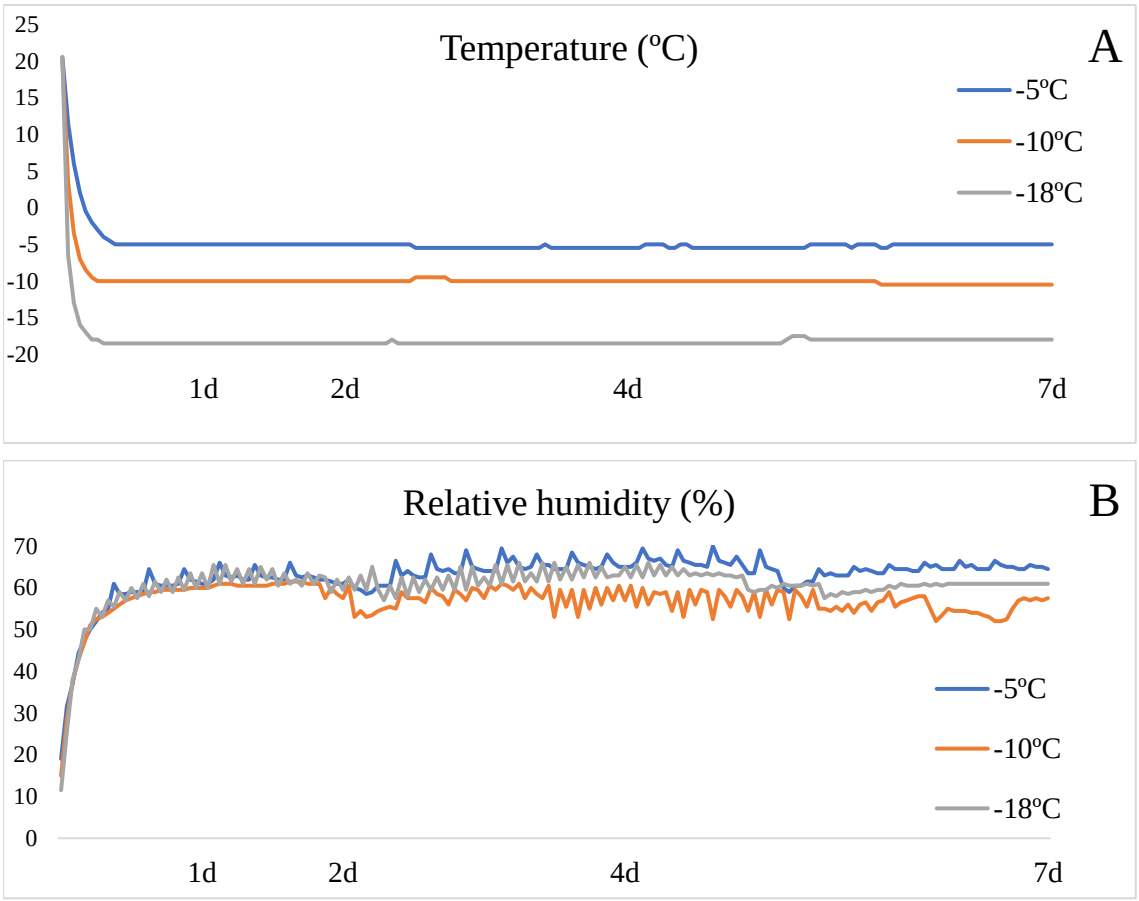


Figure 3.2. Recording of Temperatures (A) and relative humidities B) of the three freezing chambers during the treatments.

2.1.2 Testing insects

Lepidopteran pests were recorded as mixed. Emerging adults of these species were counted and removed from the punnets. At the end of the experiment, dried figs were dissected and visually inspected under a stereomicroscope, and the number of live or dead lepidopteran larvae inside was counted. The results were expressed as the percentage of insects. Another common pest of dried figs, *C. hemipterus*, was only recorded as the number of punnets infested out of the total number of punnets analysed due to its high number of individuals and rapid spread.

2.2 Experimental design 2: Quality characteristics of dried figs after the application of optimal pest control treatment

2.2.1 Plant material

High-quality dried figs (Calabacita variety), classified as Class I (UNECE, 2016), were used for this experiment. Fruit was harvested in 2021 at the research centre "Finca la Orden - CICYTEX" (Guadajira, Badajoz, Spain; 38° 85′ 19″ N, −6° 68′ 28″ W), hand-picked in a mesh of a super high-density crop system described in our previous work (Galván et al., 2021). All selected dried figs were homogeneous in size and colour, free of insects, and without visual defects. According to the results of experimental design 1, three freezing treatments were carried out at different temperatures and durations (T1: −5°C, 7 days; T2: −10°C, 2 days; T3: −20°C, 1 day), and phosphine-treated fruit was used as the control (CT). The same freezing procedure was followed as in the previous experiment, but after the treatment, the figs were defrosted for 24 h at room temperature (20°C) and then analysed. All measurements were performed in triplicate from three different punnets.

2.2.2 Physicochemical parameters

2.2.2.1 Moisture content and water activity

Moisture content and water activity (a_w) were measured using the official AOAC Method 934.01 using an a_w meter (LabMASTER-aw, Novasina AG, Lachen, Switzerland) on a homogenised mixture of 10 fruits.

2.2.2.2 Total soluble solids

The total soluble solids (TSS) content was tested in 10 chopped and homogenised fruits using an RM40 digital refractometer (Mettler Toledo, Madrid, Spain), and the results were expressed in °Brix.

2.2.2.3 Texture profile analysis

Texture profile analysis (TPA) was measured on 15 fruits using a TA.XTPlus texture analyser (Stable Micro Systems, Godalming, UK) with a cell load of 30 kg and a 70 mm cylindrical plate, following the method described by Ansari et al. (2014). The textural parameters considered in the present study were hardness (N), springiness (mm), cohesiveness (dimensionless), gumminess (N), chewiness (N mm) and resilience (dimensionless) (Bourne, 1978).

2.2.2.4 Colour objective

Skin colour was measured on two opposite sides of 15 fruits. The parameters lightness (L*), chroma (C*) and hue angle (h*) were determined using a CR-400 tristimulus colourimeter (Minolta, Tokyo, Japan) according to the CIELab system.

2.2.2.5 Sugars and organic acids

The whole dried figs from different treatments were chopped separately, and 2 g of each mixture was homogenised with 50 mL of deionised water using a homogeniser (OMNI MIXER). Sample extracts were shaken for 1 h at room temperature, filtered through a 0.22 µM filter and transferred to a vial. For sugar determination, the filtrate was diluted at 1:10 with HPLC water.

Sugar identification and quantification were determined using a high-performance liquid chromatography (HPLC) system (Agilent LC 1260 Infinity II, Waters, Milford, MA, USA) coupled to a refractive index detector (RID) (Agilent.G7162A) and with the

column temperature maintained at 60°C. Sugar separation was performed using a Rezex-RCM monosaccharides Pb^{+2} (8%) column (100 × 7.8 mm, Phenomenex Torrance, CA, USA) and HPLC water as the mobile phase with an isocratic flow rate of 0.6 mL/min. Organic acid identification and quantification were performed using an HPLC system (Agilent LC 1260) coupled to a diode array detector (DAD) (Agilent G7117C) set at 210 nm and with the column temperature maintained at 60°C. The separation of organic acids was carried out using a Rezex-ROA organic acid H^+ (8%) column (150 × 7.8 mm, Phenomenex, Torrance, CA, USA) and 0.005 N H_2SO_4 as the mobile phase with an isocratic flow rate of 0.5 mL/min. In both determinations, the injection volume was 20 μL, and the calibration curves were generated using synthetic standards purchased from Sigma-Aldrich (St. Louis, MO, USA). Sugar and organic acid concentrations were expressed in g/100 g dry weight (DW).

2.2.3 Bioactive compounds

2.2.3.1 Total phenolic content

Total phenolic compound analysis was carried out following the method described by Pereira et al. (2017) using Folin–Ciocalteu reagent. Briefly, 5 g of homogenate (n = 3) was mixed with 100 mL of an extraction solution composed of aqueous ethanol (80%) acidified with 1% HCL. After evaporation of the extract, 1 mL of the aqueous extract was used to carry out the reaction with Folin–Ciocalteu reagent, and after 1 h in the dark, the absorbance was measured at 760 nm. The total phenolic content was expressed as mg of gallic acid equivalents/100 g DW.

2.2.3.2 Total antioxidant activity

Total antioxidant activity (TAA) was quantified using the 1,1-diphenyl- 2-picrylhydrazyl radical (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

($ABTS^{\bullet+}$) methods described by Pérez-Jiménez et al. (2008). Briefly, 2 g of homogenate per treatment was extracted using 20 mL of an aqueous solution of methanol (50%) acidified with HCl at pH 2.0 and shaken for 1 h on an orbital shaker covered with ice and protected from light.

The supernatant was then centrifuged and separated. The residue was extracted again using an aqueous acetone solution (70%). Finally, both supernatants were mixed in a flask and diluted to 50 mL with Milli-Q water. The extracts were mixed with methanolic solutions of DPPH and $ABTS^{\bullet+}$, and the absorbances were measured at 515 nm at 0 and 120 min for DPPH and at 734 nm at 0 and 30 min for $ABTS^{\bullet+}$. The results were expressed as mg Trolox/100 g DW.

2.2.4 Microbiological analysis

Fifteen grams of a sample from several fruits were diluted under sterile conditions in 135 mL of peptone water (Condalab, Madrid, Spain) and homogenised using a Stomacher 400 (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Homogenates were serially diluted using peptone water, and aliquots of 0.1 mL from each dilution were plated on agar plates. Plate count agar was used to count mesophilic aerobic bacteria (incubation at 30°C for 2 days). For mould and yeast colonies, potato dextrose agar (PDA agar, Condalab, Madrid, Spain) plates acidified to pH 3.5 with a 10% (w/v) sterile tartaric acid solution were incubated at 25°C for 5 days. Microbial counts were expressed as log cfu/g.

2.2.5 Sensorial analysis

The sensory quality of the dried figs was tested using 15 previously trained panellists who carried out a descriptive sensory analysis following international standard methods (ISO 4121 -2006). Before the evaluation, the dried figs from each batch were

blanched for 60 s at 100°C. An unmarked line (scale of 10 points) was used for the panellists to test the sensory descriptors: appearance, smell, firmness, taste and flavour. Furthermore, a panel of 50 untrained panellists but regular consumers of dried figs performed a hedonic test to determine global acceptance.

2.3 Statistical analysis

The influence of freezing temperatures and the duration of treatment on insect infestation was approached with a two-way analysis of variance (ANOVA) using SPSS for Windows, 25.0. A one-way ANOVA was developed to evaluate the influence of the optimum freezing treatment (CT, T1, T2 and T3) on physicochemical parameters, total phenolic content, total antioxidant activity, microbiological load and sensory quality. The Bonferroni post hoc test was then applied to compare the mean values, with a significance level of $p \leq 0.05$. Finally, a principal component analysis (PCA) was performed to evaluate the effect of the freezing treatment on the overall quality parameters.

3. Results

3.1. Effect of different freezing treatments on insect populations

The initial infestation level of the dried figs used in the first experiment was 44.7% (22.7% for Lepidoptera and 22.0% for Coleoptera). The insect species found inside the dried figs were two pyralid moths, *P. interpunctella* (BOLD:AAB2462) and *E. ceratoniae* (BOLD:AAU4812), and one nitidulid beetle, *C. hemipterus* (BOLD:AAN6006).

The effect of different temperatures and exposure periods on different species of carpophagous insects harboured in dried figs is shown in Table 3.1. The treatment at −5°C significantly affected the mortality of Lepidoptera across exposure periods ($p < 0.05$). After 1 day, mortality reached 19.26%, rising sharply during the freezing period up to 100% after 7 days. Concerning emerging adult moths, the untreated samples showed the highest levels, with a mean value of 72.08%. In contrast, the percentage of individuals recorded at different freezing periods significantly decreased with treatment duration ($p < 0.05$), reaching 0% after 7 days. The percentage of diapausing moth larvae found in the internal cavity of fig showed no statistical differences after 1 and 2 days of treatment at −5°C compared to the untreated batch; however, after 4 days of freezing, they were no longer detected.

Regarding the treatments at −10 and −18°C throughout the freezing period, their efficiency in controlling Lepidoptera species was as good as that of the industrial application of phosphine. After 1 day of exposure at both temperatures, the mortality was 100%. No adults or larvae were found in the internal cavity of the fruit, showing significant differences *($p < 0.05$)* with the untreated batch.

Table 3.1. Effect of freezing treatment at different temperatures and durations on the insect pests in infested dried figs.

Treatments		Lepidoptera: Pyralidae			Presence of alive *C. hemipterus* (punnet)
		Mortality (%)	Emerged adults (%)	Larvae diapause (%)	
−5°C	1 d	19.26 ± 12.96^{c1}	44.38 ± 21.20^{b1}	36.35 ± 26.73^{a1}	9/10
	2 d	45.37 ± 17.39^{b1}	17.69 ± 20.64^{c1}	36.94 ± 23.83^{a1}	1/10
	4 d	94.31 ± 10.89^{a}	5.69 ± 10.89^{cd}	0 ± 0^{b}	0/10
	7 d	100 ± 0^{a}	0 ± 0^{d}	0 ± 0^{b}	0/10
	Untreated	1.14 ± 3.77^{d}	72.08 ± 31.55^{a}	26.67 ± 31.38^{a}	8/10
−10°C	1 d	100 ± 0^{a2}	0 ± 0^{b2}	0 ± 0^{b2}	1/10
	2 d	100 ± 0^{a2}	0 ± 0^{b2}	0 ± 0^{b2}	0/0
	4 d	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	0/0
	7 d	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	0/0
	Untreated	1.85 ± 5.27^{b}	75.17 ± 20.73^{a}	23.17 ± 22.09^{a}	8/10
−18°C	1 d	100 ± 0^{a2}	0 ± 0^{b2}	0 ± 0^{b2}	0/10
	2 d	100 ± 0^{a2}	0 ± 0^{b2}	0 ± 0^{b2}	0/10
	4 d	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	0/10
	7 d	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	0/10
	Untreated	0.0 ± 0^{b}	85.88 ± 16.26^{a}	14.12 ± 16.26^{a}	10/10
Phosphine		100 ± 0	0 ± 0	0 ± 0	0/10
P. Temperature		< 0.001	< 0.001	< 0.001	
P. Time		< 0.001	< 0.001	< 0.001	
*P. Temperature * Time*		< 0.001	< 0.001	< 0.001	

a, b, c Indicate significant differences ($p \leq 0.05$) among periods of the same temperature.

[1,2] Indicate significant differences ($p \leq 0.05$) among temperatures of the same period.

*Positive phosphine control was not entered into the statistical analysis.

In the −5°C treatment, living *C. hemipterus* individuals gradually decreased during the freezing period. After 1 day of treatment, the number of punnets with alive individuals was analogous to that of the untreated batch. After this time, their viability decreased sharply, requiring a minimum of 4 days at −5°C to reduce the occurrence of alive individuals to 0 in the 10 punnets tested, leading to the death of all specimens. For the other temperatures tested, −10 and −18°C, the occurrence of *C. hemipterus* was detected only after 1 day at −10°C, with a 1/10 coefficient. For the rest of the freezing periods tested, the occurrence of this species was not found under either temperature.

Thus, the shortest freezing period in which no alive insect species were found at the applied temperature was 7 days at −5°C (T1), 2 days at −10°C (T2), and 1 day at −18°C (T3). With these results, these three treatments (temperature and time duration) and one phosphine-treated (CT) control were applied to dried figs of the Calabacita variety without insect infestation to evaluate the impact on their physicochemical, microbiological and sensory quality.

3.2. Physicochemical quality of dried figs after the application of optimal pest control treatments

3.2.1. Moisture content, water activity and total soluble solids

The moisture content, a_w and TSS of dried figs after T1, T2, T3 and CT treatments are shown in Table 3.2. The moisture content and a_w values ranged from 20.56 to 24.31% and from 0.58 to 0.70, respectively. In contrast, CT showed a lower mean value for both parameters, with no significant differences compared to T3, although T1 showed significantly higher values ($p \leq 0.05$). The mean values of both parameters increased when the treatment period was longer and the freezing temperature was closer to 0°C. The TSS was also significantly affected by freezing treatments ($p \leq 0.05$), and the mean

values ranged between 79.1 and 83.41°Brix. CT showed the highest value, with 83.41°Brix, showing significant differences ($p \leq 0.05$) from the other treatments. Among freezing treatments, T1 showed the lowest value (79.1°Brix), while no significant differences ($p > 0.05$) were found between T2 (82.08°Brix) and T3 (82.24°Brix) treatments.

Table 3.2. Effect of freezing treatments on physicochemical parameters of dried figs.

Treatment	Moisture Content (%)	a_w	TSS °Brix	Glucose (g/100 g DW)	Fructose (g/100 g DW)	Citric acid (g/100 g DW)	Malic acid (g/100 g DW)
CT*	20.56^{c+}	0.58^{b}	83.41^{a}	39.09^{a}	38.38^{a}	0.28^{a}	1.63^{a}
T1	24.31^{a}	0.70^{a}	79.1^{c}	35.06^{b}	33.84^{b}	0.26^{ab}	1.52^{a}
T2	22.26^{b}	0.61^{b}	82.08^{b}	32.36^{c}	31.62^{c}	0.24^{b}	1.22^{b}
T3	21.35^{bc}	0.61^{b}	82.24^{b}	30.87^{c}	29.51^{c}	0.24^{b}	1.02^{b}
P	< 0.001	0.001	< 0.001	< 0.001	<0.001	0.015	<0.001

$^{+}$In each column, different lowercase letters indicate a significant difference at $p < 0.05$ (Tukey's test).

*CT: Phosphine-treated figs; T1: −5°C, 7 days; T2: −10°C, 2 days; T3: −18°C, 1 day.

3.2.2. Sugar and organic acid content

Table 3.2 shows the concentrations of sugars and organic acids after the different freezing treatments. Glucose and fructose were the dominant sugars detected. In all samples analysed, glucose levels were higher than fructose levels, with mean values of 34.3 and 33.3 g/100 g DW, respectively. Specifically, glucose values ranged from 30.87 (T3) to 39.09 g/100 g DW (CT), whereas fructose values ranged from 29.51 (T3) to 38.38 g/100 g DW (CT). The content of both sugars was significantly ($p \leq 0.05$) affected by the freezing treatment applied in the same manner. CT showed significantly higher values, while T2 and T3 treatments showed the lowest values ($p \leq 0.05$).

The organic acid content was also significantly affected by the freezing treatment. In general, malic acid levels were around 5-fold higher than citric acid in all samples analysed. Malic acid levels ranged from 1.02 to 1.63 g/100 g DW, and the T2 and T3 treatments had significantly lower values ($p \leq 0.05$) than the CT and T1 treatments. For citric acid, the levels ranged between 0.24 (T2 and T3) and 0.28 g/100 g DW (CT), with significant differences ($p \leq 0.05$) between the CT and freezing treatments.

3.2.3. Colour objective

The colour parameters evaluated are shown in Table 3.3. L* ranged from 57.91 to 59.52, C* from 34.99 to 37.95 and h* from 72.70 to 73.49. CT and T1 reached the maximum and minimum mean values for all colour parameters tested. However, significant differences ($p \leq 0.05$) between treatments were only found for C*, while L* and h* parameters remained unaffected by the freezing treatment ($p > 0.05$). T2 and T3 treatments, shorter duration, and lower temperatures did not produce significant changes in dried fig skin colour compared to CT. In contrast, T1 led to a slight darkening of skin colour, showing a significant decrease ($p < 0.05$) in the C* parameter compared to CT.

Table 3.3. Effect of freezing treatments on textural parameters (TPA) and colour parameters of dried figs.

		CT *	T1	T2	T3	*P*
Colour	L*	59.52 ± 5.04	57.91 ± 5.36	59.29 ± 5.30	59.28 ± 4.70	0.606
	C*	37.95 ± 3[a+]	34.99 ± 3.5[b]	37.08 ± 3.19[ab]	36.44 ± 2.9[ab]	0.004
	h*	73.49 ± 2.53	72.70 ± 2.61	72.72 ± 2.34	73.08 ± 2.18	0.545
TPA	Hardness (N)	7.98 ± 2.86[ab]	3.96 ± 1.88[c]	7.07 ± 2.66[b]	8.80 ± 3.69[a]	< 0.001
	Springiness (mm)	0.78 ± 0.10	0.80 ± 0.06	0.81 ± 0.05	0.85 ± 0.28	0.177
	Cohesiveness	0.69 ± 0.05	0.72 ± 0.05	0.70 ± 0.06	0.71 ± 0.06	0.223
	Gumminess (N)	5.39 ± 1.99[ab]	2.82 ± 1.31[c]	4.96 ± 1.85[b]	6.24 ± 2.73[a]	< 0.001
	Chewiness (N x mm)	4.28 ± 1.69[b]	2.25 ± 1.02[c]	4.01 ± 1.56[b]	5.30 ± 2.68[a]	< 0.001
	Resilience	0.21 ± 0.02[b]	0.23 ± 0.02[a]	0.21 ± 0.02[b]	0.20 ± 0.02[b]	< 0.001

[+]In each row, different lowercase letters indicate a significant difference at $p < 0.05$ (Tukey's test).

[*]CT: Phosphine-treated figs; T1: −5°C, 7 days; T2: −10°C, 2 days; T3: −18°C, 1 day.

3.2.4. Textural parameters

The textual parameter values evaluated are shown in Table 3.3. Among them, the treatments did not significantly affect elasticity or cohesiveness ($p > 0.05$). Nevertheless, the other four tested parameters, hardness, gumminess, chewiness and resilience, showed significant differences ($p \leq 0.05$) among treatments. Specifically, T1 treatment caused the most significant alteration in the textural traits of the dried figs, with significant differences ($p \leq 0.05$) in hardness, gumminess, chewiness and resilience compared to phosphine-treated dried figs. In contrast, the T2 and T3 treatments did not alter any textural traits compared to phosphine-treated dried figs, except for chewiness, which was significantly higher in the T3 treatment.

3.2.5. Total phenolic content and antioxidant capacity

The total phenolic content and total antioxidant activity (TAA) in dried figs from the treatments tested are shown in Table 3.4. The phenolic content ranged from 90.43 (T1) to 107.23 (T2) mg gallic acid/100 g DW, with the dried figs from T2 showing the highest concentration and being significantly ($p \leq 0.05$) different from the other treatments. Concerning TAA, DPPH values ranged from 82.58 (T1) to 95.87 (T3) mg Trolox/100 g DW. T2 and T3 treatments showed significantly ($p \leq 0.05$) higher DPPH values than the phosphine and T1 treatments. The TAA shown by the ABTS method was similar to DPPH, with significant differences ($p \leq 0.05$) between T1 and the other treatments. Dried figs frozen for a longer time (T1) showed a lower content of total phenolic compounds and TAA, while treatments with shorter freezing periods (T3 and T4) better preserved their levels.

Table 3.4. Effect of freezing treatment on bioactive compounds of dried figs.

Treatment	DPPH (mg Trolox/100 g DW)	ABTS (mg Trolox/100 g DW)	Total phenols (mg gallic acid/100 g DW)
CT*	89.1^{ab}	94.55^{a}	91.42^{b}
T1	82.58^{b}	80.56^{b}	90.43^{b}
T2	90.03^{a}	98.60^{a}	107.23^{a}
T3	95.87^{a}	98.17^{a}	95.22^{b}
P	0.002	0.000	0.001

+In each column, different lowercase letters indicate a significant difference at $p < 0.05$ (Tukey's test).

*CT: Phosphine-treated figs; T1: −5°C, 7 days; T2: −10°C, 2 days; T3: −18°C, 1 day.

3.2.6. Microbiological analysis

The counts of the microbial groups tested are shown in Figure 3.3. The freezing treatments did not affect ($p > 0.05$) the microbial counts of aerobic mesophilic bacteria, yeasts or moulds (Figure 3.3). The mean values recorded for all treatments were 3.6, 3.1 and 2.9 log cfu/g, for total mesophilic bacteria, moulds and yeasts, respectively.

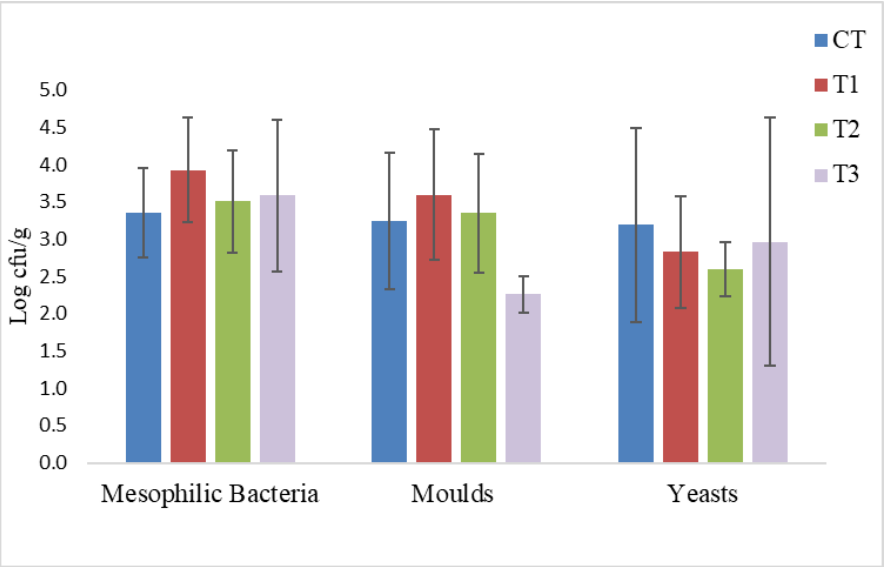


Figure 3.3. Microbial counts ± SD (log cfu/g) of dried figs from phosphine (CT) and freezing treatments (T1: −5°C for 7 days; T2: −10°C for 2 days; T3: −18°C for 1 day).

3.2.7. Sensory analysis

The statistical analysis of the sensory descriptors showed no significant differences ($p > 0.05$) between the freezing and control treatments tested, except for the firmness parameter, which was significantly lower after applying the T1 treatment (Figure 3.4). Fig skin colour was visually evaluated by each panellist on a scale from light brown (1) to very dark brown (10), with the mean of all treatments being golden brown (2.8). The T1 treatment scored the highest mean values for smell and flavour parameters, with no significant differences from the other treatments. Smell and flavour defects were almost absent, as their mean score was less than 1 on a scale of 1 to 10 for all treated and phosphine-treated dried figs. Regarding overall acceptability, freeze-dried figs (T1, The T2 and T3) obtained better mean values than phosphine-treated figs (CT), with extreme values of 6.53 and 6.99 for the phosphine-treated and T1 treatment, respectively.

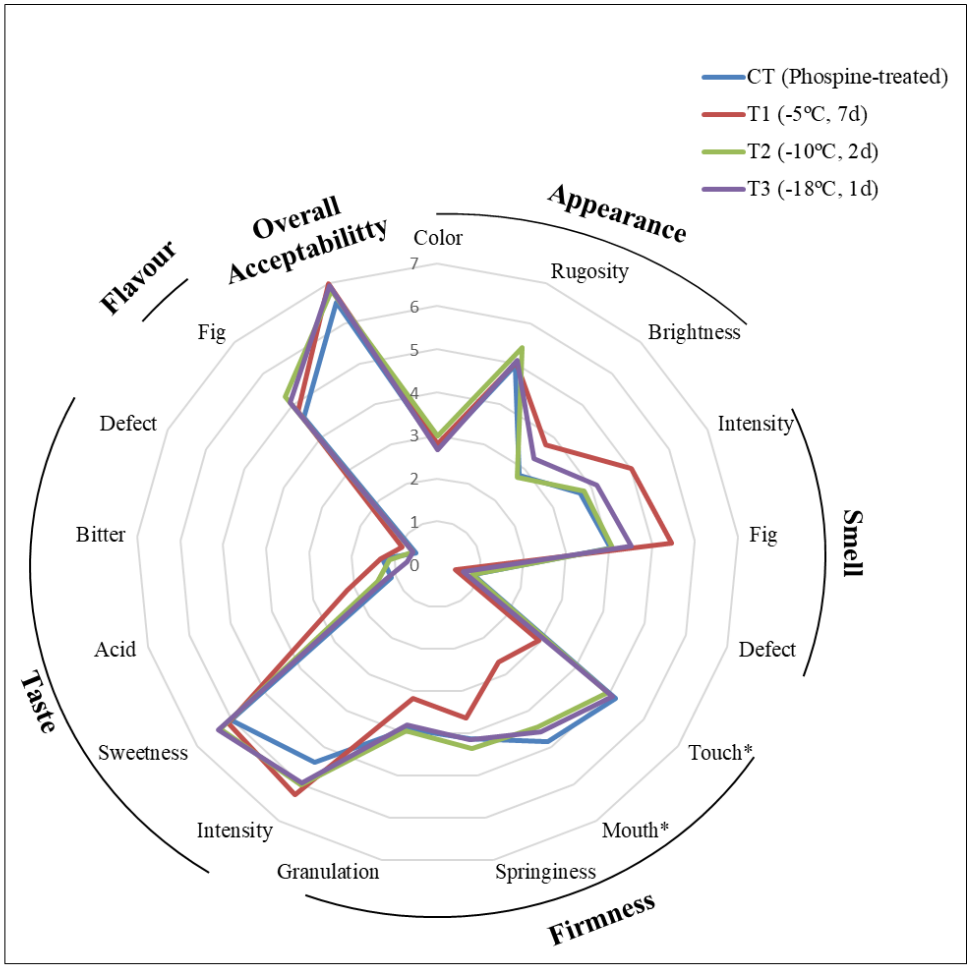


Figure 3.4. Evaluation of descriptive sensory parameters and overall sensory quality of dried figs after application of the freezing treatments (blue colour: untreated batch (CT); red colour: T1: −5°C for 7 days; green colour: T2: −10°C for 2 days; purple colour: T3: −18°C for 1 day). * Indicate significant differences ($p \leq 0.05$) among treatments.

3.2.8. Multivariate analysis

Figure 3.5 shows the PCA loading and score plots of the studied parameters and applied treatments. Most of the parameters studied were mainly explained by the first component (PC1, 56.1% variability), which places moisture content, a_w, mesophilic bacteria and mould counts, overall acceptability, and textural parameters, such as resilience and cohesiveness, on the negative axis (Fig. 3.4A) related to figs from T1 treatment (Fig. 3.4B). However, T2 and T3 treatments differed to a lesser extent from phosphine-treated dried figs (CT), with them all found in the positive part of the axis

linked to textural parameters (hardness, gumminess, chewiness and elasticity), colour parameters, bioactive compounds, yeast count and TSS. Other parameters such as sugar and organic acid content were mainly explained by the second principal component (PC2, 34.0% variability) related to phosphine-treated figs (CT).

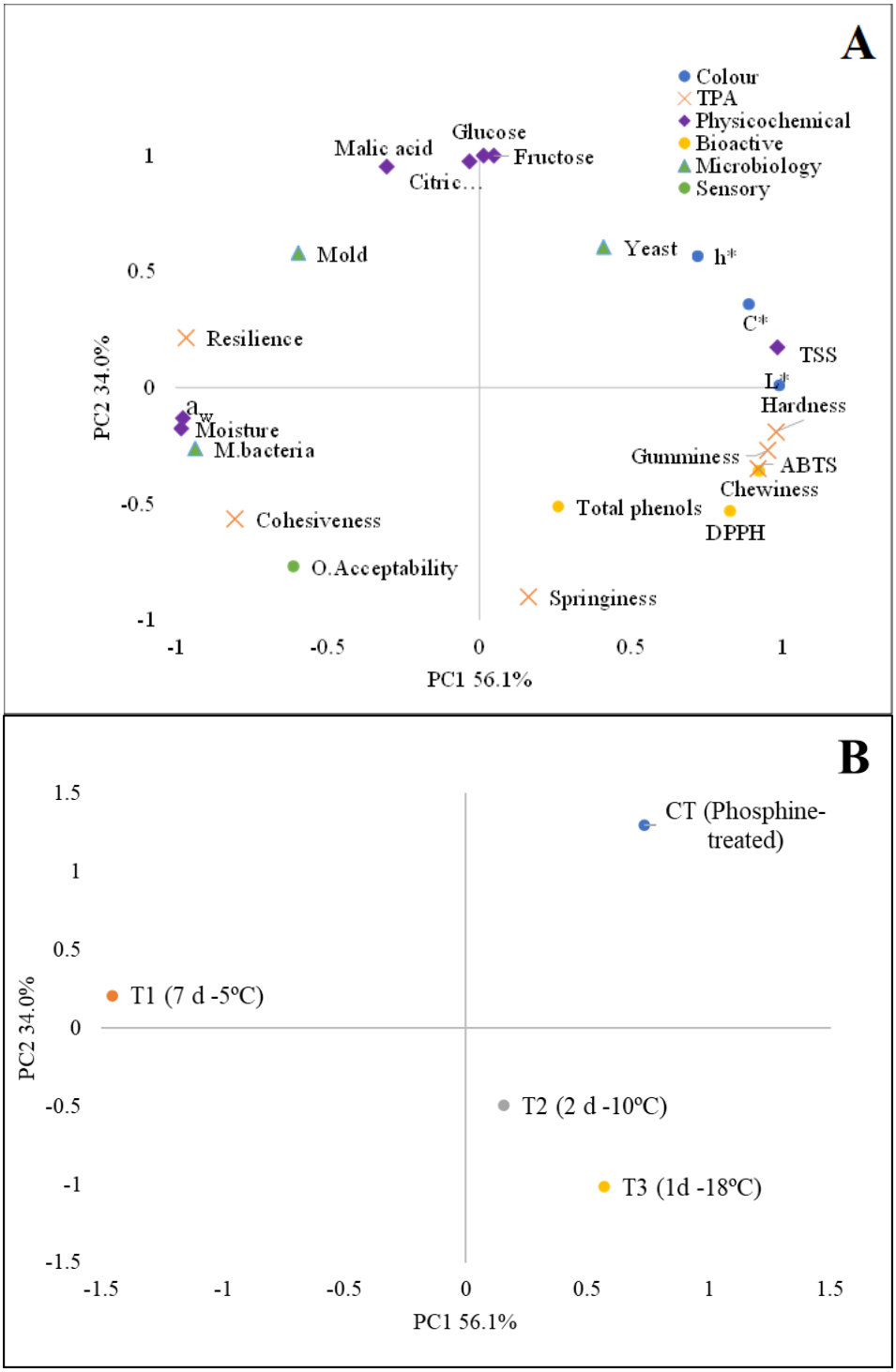


Figure 3.5. Projection of samples grouped according to colour, texture parameters (TPA), physicochemical, bioactive compounds, microbiology and sensory (A) and treatment applied (B) in the space defined by the first two components (PC1/PC2).

4. Discussion

The use of freezing temperatures is a widespread and effective treatment for the post-harvest disinfestation of stored foods (Donahaye et al., 1995; Burks et al., 2000; Fields et al., 2012; Andreadis and Athanassiou, 2017; Ben-Amor et al., 2019). Donahaye et al., 1995;It is effective against insect species that have developed insecticide resistance; no licences are required for its use, and applicators and consumers are not exposed to chemical hazards (Andreadis and Athanassiou, 2017). For their integration into the dried fig industry, it is first necessary to optimise the minimal storage period and temperature needed to control the specific pests of dried figs and to understand how these treatments can affect fruit quality. In this sense, the minimum freezing conditions required were T1: 7 days at −5°C, T2: 2 days at −10°C and T3: 1 day at −18°C to provide 100% mortality of the target insect pests harboured on dried figs. Donahaye et al. (1991) for *C. hemipterus* control and Athanassiou et al. (2018) for *P. interpunctella* control have shown results in terms of temperature and duration similar to those of our experiment. Different studies have also shown efficacy in controlling *E. ceratoniae* with exposure to −18°C for different storage periods in products similar to dried figs (Ben-Amor et al., 2019; Lallouche et al., 2017).

In the world trade of dried figs, both the safety and quality of the final product play a crucial role (Aksoy, 2019). The most critical parameters that determine quality are colour, texture, size, visual appeal, flavour, nutrients, microbial load, moisture, water activity ($_a$w), chemical stability, absence of insects, pests and off-odours (Perera, 2005; Aksoy et al., 2008).

Depending on the environmental storage conditions, dried figs may absorb or desorb water, impacting their quality (Hssaini et al., 2022). During freezing treatments,

the moisture content increased but never exceeded the 26% moisture content limit set by the DDP-14 standard for commercial and marketing quality control of dried figs (UNECE, 2016). The average relative humidity in each of the three chambers during the first 48 h after reaching the treatment temperature was 50–70% (Table S1), while in the control, it was ≈ 30%. Similar to moisture content, water activity (a_w) increases as the relative humidity of the environment increases, thus affecting its microbiological quality and food safety (Moraga et al., 2011; Lee et al., 2015a; Şen, 2022). For the food safety of dried figs, an a_w value of less than 0.65 is required to reduce the growth of moulds and mycotoxin formation (Perera, 2005; Galván et al., 2023). For treatment 1, an a_w of 0.7 was recorded, possibly due to prolonged exposure to high relative humidity (60–70%). To maintain dried fig quality, controlling the ambient RH% during postharvest handling is essential (Pixton and Warburton, 1976), even during freezing treatment, as shown in our work. Figs show their highest TSS in tree-ripe fruit, not increasing at later stages (Şen et al., 2017). Ben-Amor et al. (2019) reported no changes in the TSS in palm dates after treatment at −18°C for 50 h. However, the moisture content is closely related to TSS in figs (Silva et al., 2013). The increase in moisture content could explain the decrease in TSS after freezing treatments were applied in our study. The results obtained were within the range described by Galván et al. (2023) for dried figs of the same variety under study.

Figs are characterised by being very rich in reducing sugars, with glucose and fructose being the main ones (Vinson, 1999; Genna et al., 2005; Wojdyło et al., 2016). Jemni et al. (2019) reported a decrease in glucose and fructose after freezing palm dates at different temperatures for 10 months. In contrast, monosaccharides increased on dates with a shorter freezing time (50 h at −20°C) (Ben-Amor et al., 2019). The current experiment showed that freezing dried figs reduced the amount of both sugars. These results agree with those obtained by Wojdyło et al. (2016), who also found an increase in

sugar content in treated figs compared to phosphine-treated figs. The same behaviour as sugars was observed for organic acids. The organic acids are more diluted in dried fig samples with a higher moisture content (Slatnar et al., 2011).

Colour and hardness are among the most important attributes of dried figs (Perera, 2005; Şen et al., 2010; Galván et al., 2023). Colour changes after freezing may occur due to the oxidation of chemical compounds, such as water-soluble polyphenols. Experiments showed that figs stored longer (T1) were duller, with a lower C* coordinate (Skrede, 1996). Regarding firmness, studies on the freezing preservation of fresh figs (Ertan et al., 2020) have shown that firmness decreases after 60 and 300 days of freezing, similar to our study. In contrast, firmness is maintained or even increased on fruit dates (Ben-Amor et al., 2019; Jemni et al., 2019). This could be due to the high correlation between the hardness or softness and the moisture content of the fruit (Şen et al., 2009). Ansari et al. (2014) reported that as fruit moisture content increased, different textural attributes, such as hardness, chewiness and gumminess, decreased.

Fig health benefits are well known due to their polyphenol content and total antioxidant activity (Pereira et al., 2017). A previous freezing study of fresh figs (–18°C, 4 months) showed decreased polyphenol content and total antioxidant activity (Petkova et al., 2019). However, in our study, only the longest freezing time (T1) showed decreased antioxidant activity, while polyphenol levels remained unchanged. Similar behaviour was observed by Ben-Amor et al. (2019) in palm dates after freezing (−18°C, 50 h). These polyphenols are secondary metabolites that plants and fruit produce to respond to abiotic or biotic stress, including dramatic temperature changes (Medda et al., 2020).

During the production of dried figs, several factors can trigger the propagation of microorganisms (Gilbert and Senyuva, 2008). Regarding freezing temperatures, these

limit the growth of microorganisms, but most are not destroyed and will grow as quickly as food is defrosted (Geiges, 1996). Counts after freezing treatments were similar to those described by Galván et al. (2022a) under the exact handling and harvesting conditions; freezing did not affect mould, yeast or mesophilic bacteria counts.

Sensory evaluation of foodstuffs is an important parameter, as it indicates the sensory descriptor attribute, which showed no differences between the control and frozen figs, except for some firmness parameters. As expected, the mouth and touch firmness scores of the T1 treatment figs were lower than those of the others, as shown by the TPA results (Table 3). Overall acceptability was good for all treatments, with an average score of 6.8. This result shows that freezing treatments, in addition to pest control, improve the acceptability of the product.

5. Conclusions

Results have shown that freezing can be an alternative to conventional phosphine treatments for pest control in dried figs. All three treatments applied, 1 day at −18°C, 2 days at −10°C and 7 days at −5°C were suitable to control the most common pests occurring in dried figs. Furthermore, the 7-day treatment at −5°C positively impacted product sensory characteristics, while from a bioactive compound point of view, the 2-day treatment at −10°C better maintained the bioactive characteristics of the fruit, even showing significant differences concerning phosphine-treated figs. Therefore, freezing is an effective tool for the dried fig industry to control pests during storage and marketability and to contribute to reducing the use of synthetic chemical products.

CHAPTER 4

CHAPTER 4

Evaluation of Carifend® for the control of larvae of major stored-product species.

1. Abstract

In the present study, Carifend® (BASF AG, Ludwigshafen, Germany) an alpha-cypermethrin-coated net was evaluated in laboratory conditions against larvae of major stored-product insect species. This experiment was conducted using petri dishes, in which the insecticide-treated net was placed at the bottom, whereas two additional series of dishes were employed as controls, one with a net but no insecticide, and the other without net. Five major insect species of stored products were tested, i.e. *Tribolium confusum*, *Tribolium castaneum*, *Alphitobius diaperinus*, *Tenebrio molitor* and *Ephestia kuehniella.* Larvae of these species were exposed to the treatments for 1 and 7 d, then they were transferred to untreated dishes, where mortality was recorded immediately and 7 d later. In all species, an increase in the duration of initial contact with Carifend® and duration of post-exposure increased the level of mortality. Among the species tested, *T. castaneum* was the least susceptible to Carifend®, with 88.9% mortality after 7 d of contact, while mortality for *A. diaperinus* larvae reached 100% under the same conditions. The results of this study show that Carifend® is effective against larvae of a wide range of storage insect pests, making it a valuable tool for the protection of durable food commodities.

Keywords: alpha-cypermethrin, pest control, stored-products insects

2. Materials and methods

The experimental design is shown graphically in Figure 4.1.

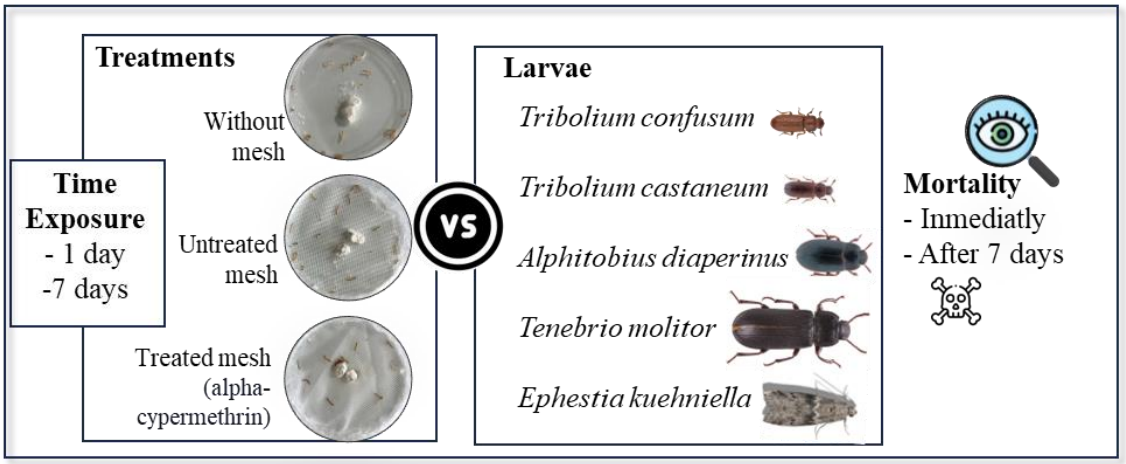


Figure 4.1. Experimental design.

2.1 Insects

All insect cultures were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture Crop Production and Rural Environment, University of Thessaly. The conditions for rearing were 25ºC, 55% relative humidity (r.h.) and continuous darkness. The rearing medium for *T. castaneum*, *T. confusum* and *E. kuehniella* was wheat flour, while for *T. molitor* and *A. diaperinus* the medium was wheat bran. Mixed sex larvae, with their age between the third and fifth instar were used in all of the trials.

2.2 Formulation and unit preparation

Carifend® formulation is a net (40 denier with mesh size 625 knots/in^{-2}) containing alpha-cyphermethrin at a concentration of 163.2 mg m^{-2} (Rumbos et al., 2018; Athanassiou et al., 2019; Paloukas et al., 2020; Agrafioti et al., 2021; 2023). This net and an identical but non insecticide-treated net (positive control) were supplied by BASF AG (Ludwigshafen, Germany).

Standard plastic Petri dishes were used for this experiment, with a 9 mm diameter Carifend® net circle fitted with silicone at the bottom (59.4 cm) of each dish. Two additional sets of plates were used as controls, one with untreated net (positive control) and one without net (negative control). The inner vertical walls of the plates were coated with Fluon (polytetrafluoroethylene preparation, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), to prevent insects from escaping.

2.3 Experimental design

Twenty larvae of each species were placed in Petri dishes, using different plates per species, and exposed to treatment and controls during 1 and 7 d. A small quantity of wheat flour (1.0 ± 0.1 g) was placed in the centre of the dishes (Agrafioti et al. 2021). After each exposure to each treatment, the immediate mortality effect was measured; insects were considered dead if they did not move after being disturbed with a fine brush. All individuals that remained alive, were transferred to empty petri dishes with a small amount of wheat flour. The delayed mortality effect was counted after 7 d. Three dishes were used for each exposure and treatment combination, while the whole trial was repeated three times (3 replicates x 3 subreplicates = 9 petri dishes for each combination). All bioassays were carried out in chambers with the same rearing conditions specified earlier, i.e., 25ºC, 55% r.h. and continuous darkness.

2.4 Data analysis

Percentage of mortality for each insect species were subjected to three-way ANOVA Fit Repeated Measures Procedure, in order to identify the differences between treatments, exposure of the same treatment and time after exposure, using the SPSS software package for Windows version 22.0 (SPSS, Inc., Chicago, IL, USA). Bonferroni

post-hoc test was applied to compare the mean values obtained, and the significance was set at $P \leq 0.05$.

3. Results

3.1. *Tribolium castaneum*

All main effects and associated interactions were significant (Table 4.1). After 1 d of exposure, mortality was low and did not exceed 13.0, 7.3, and 5.6% for Carifend®, without net, and untreated net, respectively (Figure 4.2). In contrast, a longer exposure period of 7 d with Carifend® net, resulted in a noticeable increase in immediate mortality rates (37.2%), which further escalated to 88.9% for delayed mortality. The mortality rates of Carifend® were significantly different from both controls, except for the immediate mortality after 1 d of treatment.

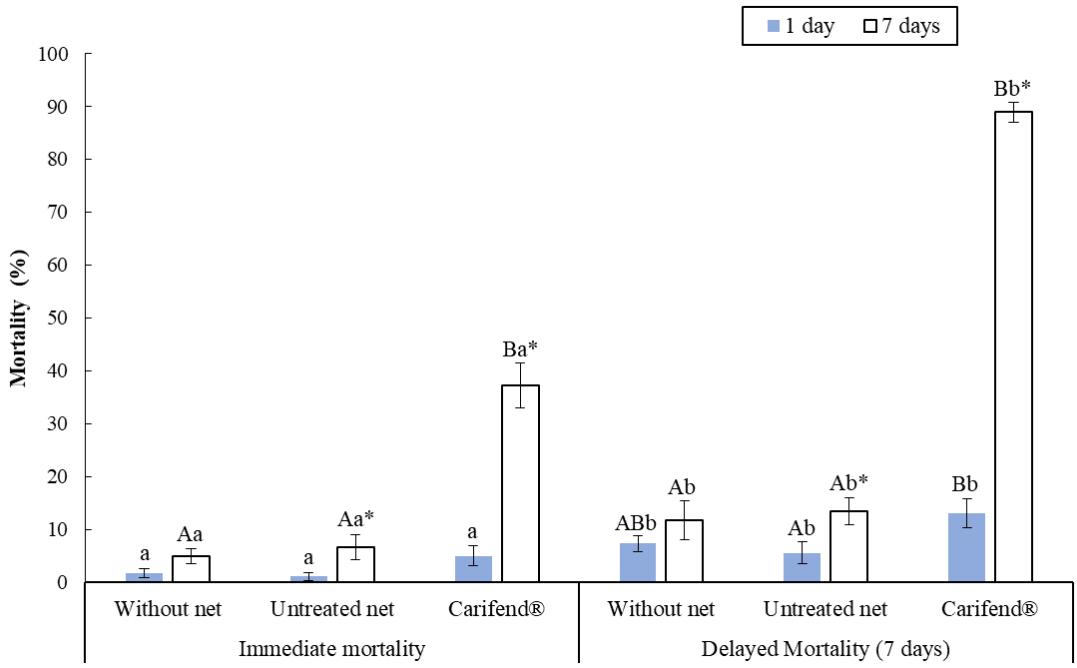


Figure 4.2. Mean immediate and delayed mortality (% ± SE) of *Tribolium castaneum* larvae exposed for 1 and 7 d to dishes without net, dishes with untreated net and dishes with Carifend® (with the delayed effect set at 7 d). Different capital letters, different lowercase letters and asterisk show significant differences ($p \leq 0.05$) among treatments, time after exposure and exposure interval under the same treatment, respectively.

Table 4.1. Repeated measures ANOVA parameters for main effects and associated interactions for mortality levels of *Tribolium castaneum, Tribolium confusum, Alphitobius diaperinus, Tenebrio molitor and Ephestia kuehniella* larvae (error df = 48).

Source		*T. castaneum*		*T. confusum*		*A diaperinus*		*T. molitor*		*E. kuehniella*	
		Mortality		Mortality		Mortality		Mortality		Mortality	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
All between											
Intercept	1	418.7	<0.001	1963.3	<0.001	2168.0	<0.001	1772.0	<0.001	906.5	<0.001
Treatment	2	151.3	<0.001	45.0	<0.001	1904.8	<0.001	1748.0	<0.001	114.0	<0.001
Exposure	1	181.0	<0.001	548.0	<0.001	1230.4	<0.001	620.7	<0.001	223.2	<0.001
Treatment x Exposure	2	103.8	<0.001	8.9	<0.001	1055.6	<0.001	620.7	<0.001	22.3	<0.001
All within interaction											
Time after exposure	1	160.5	<0.001	292.1	<0.001	66.3	<0.001	112.7	<0.001	302.6	<0.001
Time after exposure x Treatment	2	53.1	<0.001	3.5	0.039	34.1	<0.001	105.5	<0.001	5.4	0.008
Time after exposure x Exposure interval	1	51.4	<0.001	83.8	<0.001	13.7	<0.001	98.6	<0.001	3.0	0.087
Time after exposure x Trearment x Exposure interval	2	40.7	<0.001	8.9	<0.001	32.1	<0.001	98.7	<0.001	32.1	<0.001

3.2 Tribolium confusum

All effects and interactions studied were significant for mortality (Table 1). The mean delayed mortality was 66.9 and 68.9% after 7 d of exposure for dishes without net and with untreated net, respectively, suggesting high vulnerability of *T. confusum* (Figure 4.3). In the case of the dishes treated with Carifend®, there was a significant increase in mortality even after 1 d of exposure, reaching a delayed mortality of 52.8%. All treatments showed significant differences between immediate and delayed mortality.

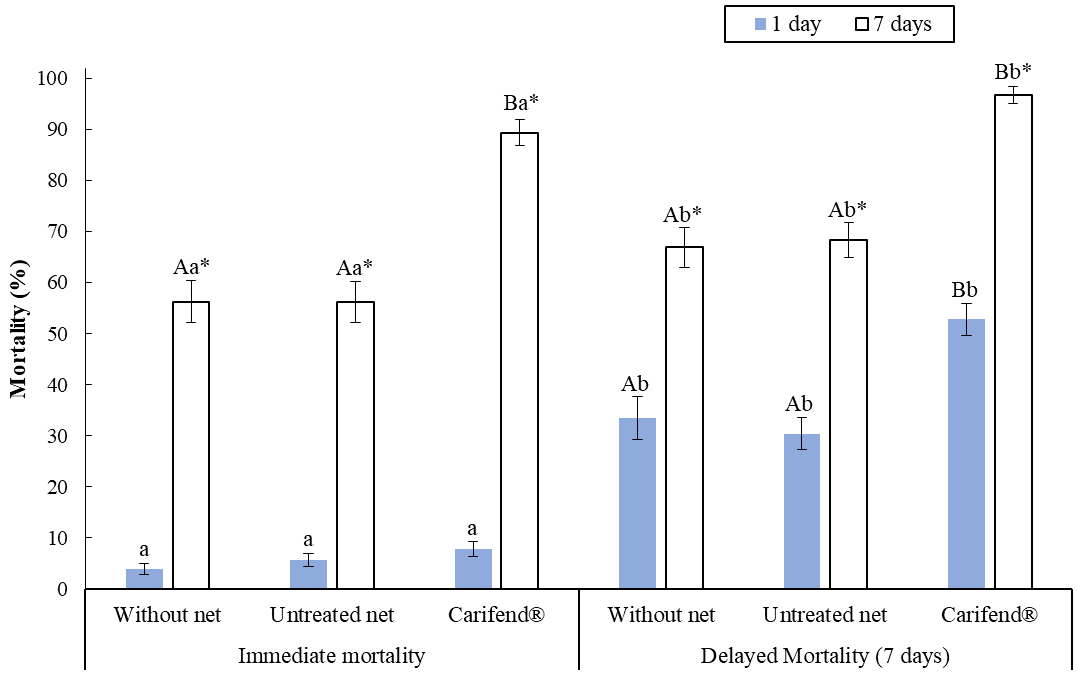


Figure 4.3. Mean immediate and delayed mortality (% ± SE) of *Tribolium confusum* larvae exposed for 1 and 7 d to dishes without net, dishes with untreated net and dishes with Carifend® (with the delayed effect set at 7 d). Different capital letters, different lowercase letters and asterisk show significant differences ($p \leq 0.05$) among treatments, time after exposure and exposure interval under the same treatment, respectively.

3.3. Alphitobius diaperinus

All main effects and associated interactions were significant (Table 1). The immediate mortality rates for all three treatments were 0% after 1 d of exposure (Figure 4.4). Delayed mortality rates were less than 1% for both control treatments, but reached 28.3% for the Carifend® treatment. Interestingly, after 7 d of exposure to Carifend®, the immediate and delayed mortality rates were exceptionally high at 96.1% and 100% respectively, which was the highest among the species studied. This contrasted with the control treatments, on which mortality was low for both exposures.

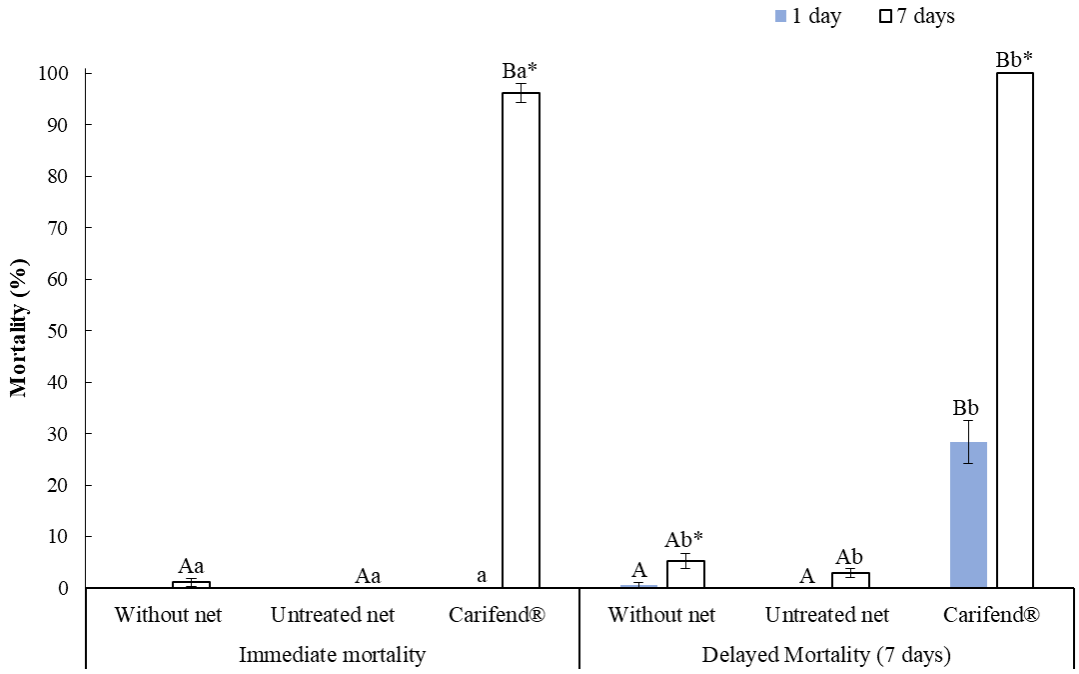


Figure 4.4. Mean immediate and delayed mortality (% ± SE) of *Alphitobious diaperinus* larvae exposed for 1 and 7 d to dishes without net, dishes with untreated net and dishes with Carifend® (with the delayed effect set at 7 d). Different capital letters, different lowercase letters and asterisk show significant differences ($p \leq 0.05$) among treatments, time after exposure and exposure interval under the same treatment, respectively.

3.4. Tenebrio molitor

All effects and interactions studied were significant (Table 1). The effect of the treatments on this species was very similar to those that were recorded in the case of *A. diaperinus* (Figure 4.5). Mortality rates were particularly low in both controls, not exceeding 0.6%. Moreover, Carifend® caused the highest immediate mortality (96.7%) of the species tested after 7 d of exposure. One week after the end of exposure to Carifend®, mortality rates increased to 49.4% and 97.8% for exposures of 1 and 7 d respectively.

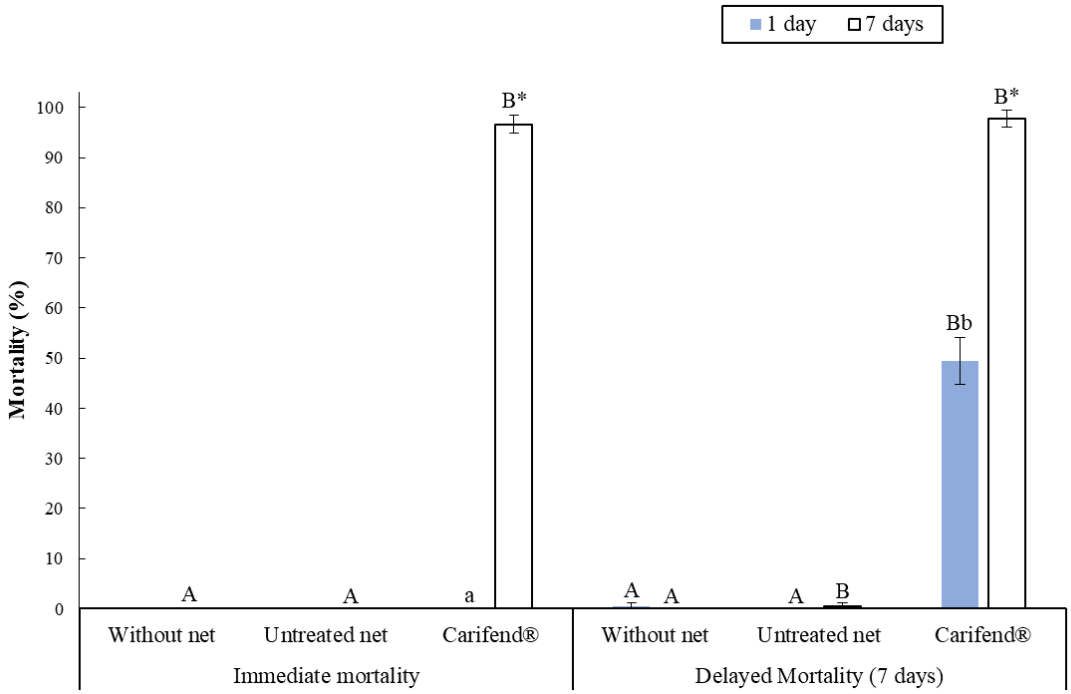


Figure 4.5. Mean immediate and delayed mortality (% ± SE) of Tenebrio molitor larvae exposed for 1 and 7 d to dishes without net, dishes with untreated net and dishes with Carifend® (with the delayed effect set at 7 d). Different capital letters, different lowercase letters and asterisk show significant differences ($p \leq 0.05$) among treatments, time after exposure and exposure interval under the same treatment, respectively.

3.5. Ephestia kuehniella

All main effects and associated interactions were significant, with the exception of Time after exposure x Exposure interval. As with the other species mentioned above, immediate mortality after 1 d of exposure was low, reaching 0.6, 2.3 and 2.2% for dishes without net, untreated dishes and dishes with Carifend®, respectively (Figure 4.6). In comparison with all species tested here, *E. khueniella* larvae reached the highest delayed mortality at 1 d exposure to Carifend® (58.9%), reaching 93.9% with the increase of the exposure time to 7 d.

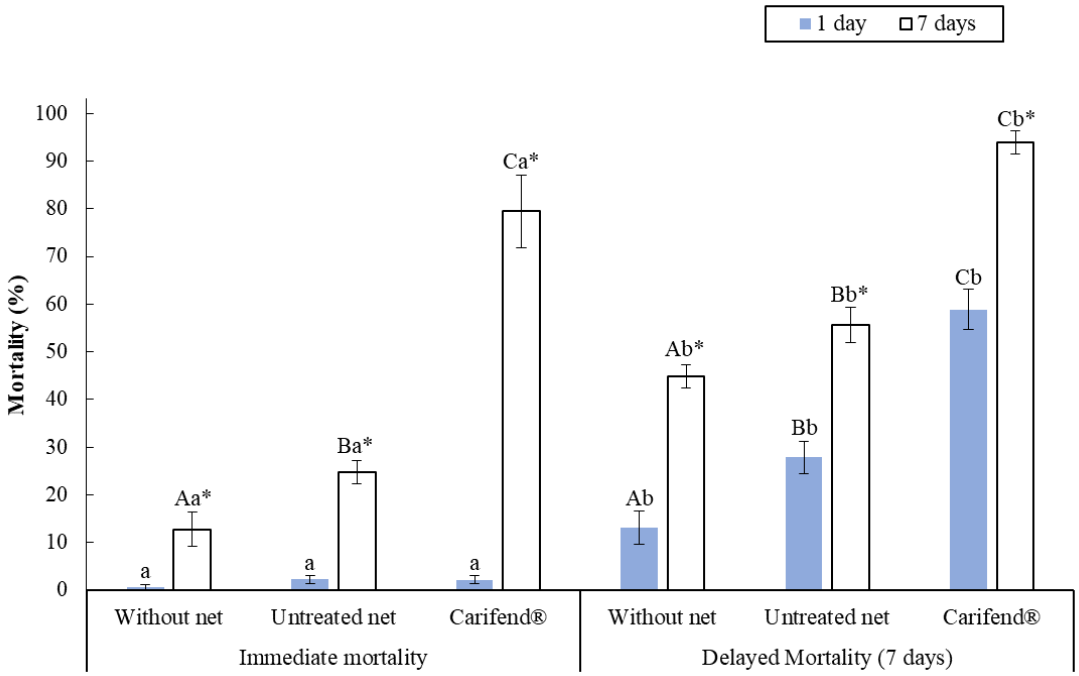


Figure 4.6. Mean immediate and delayed mortality (% ± SE) of *Ephestia kuehniella* larvae exposed for 1 and 7 d to dishes without net, dishes with untreated net and dishes with Carifend® (with the delayed effect set at 7 d). Different capital letters, different lowercase letters and asterisk show significant differences ($p \leq 0.05$) among treatments, time after exposure and exposure interval under the same treatment, respectively.

4. Discussion

Our findings clearly indicate that Carifend® demonstrated increased efficacy in controlling larvae across a wide range of stored-product insect species, with varied responses observed among them to the exposure to the treatment. The experiment evaluated both immediate and delayed mortality rates, revealing distinct patterns of susceptibility among the different species. Furthermore, our results revealed that prolonged exposure to Carifend® net resulted in a notable increase in both immediate and delayed mortality rates for several species, suggesting a consistent trend across different insects. However, a one-day exposure resulted in very low levels of mortality for all the tested species. Similar findings have been documented in prior research, wherein zero or extremely low mortality rates were reported when insects were subjected to one day or less of exposure to the treatment (Agrafioti et al., 2023, Paloukas et al., 2020). This consistency in outcomes across studies underscores the trend of minimal immediate impact on insect mortality with short-term treatment exposure.

Observable mortality was noted in the experimental dishes designated as positive and negative controls, with a considerable level of mortality observed in certain species. The observed phenomenon of elevated control mortality in petri dishes, commonly used as experimental units, has been documented in several prior studies. For instance, Athanassiou et al. (2013) reported a notable increase in control mortality after 7 days for *O. surinamensis* and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) adults in untreated dishes. Similarly, Rumbos et al. (2018) observed a significant level of adult mortality in dishes with a net similar to our positive control, resembling a scenario where the stress induced by restricting the movement of insects within a relatively confined space may have contributed to the recorded mortality. In the majority of cases, exposure to the untreated net did not lead to significantly different results from the

control. This observation underscores the importance of the insecticidal treatment in influencing outcomes, suggesting that the chemical intervention plays a more decisive role than the presence of the net in achieving the desired effects. These results align with previous research, which found no significant impact of the untreated net on insect mortality (Rumbos et al., 2018; Paloukas et al., 2020; Agrafioti et al. 2023). Specifically, Paloukas et al. (2020) assessed the effectiveness of Carifend® against adults of various stored product species, including *T. castaneum* and *T. confusum*, which are species tested in our research. They also evaluated the untreated net and reported that, for any of the species examined, the mortality at the untreated net was significantly different from the control. The present research adds valuable data, underlining that the net does not exhibit lethal effects on the larval developmental stage of the aforementioned species. Notably, the only instance where the effects of the untreated nets differed significantly from the control was observed in *E. kuehniella*, the only Lepidopteran species examined in our study. This suggests susceptibility of larvae in the Lepidoptera family to both treated and untreated nets, possibly attributed to distinctions in larval ecdysis, chitin composition, and movement patterns. However, further investigation is required to verify the reason behind this statement. Furthermore, although the mortality of the untreated nets is not significantly different from the control, divergent research suggests that they can function as formidable physical barriers, impeding insect movement and potentially providing protection even after the dissipation of the insecticide (Morrison et al., 2018; Wilkins et al., 2020, 2021; Agrafioti et al., 2021; Gerken et al., 2021). Consequently, the implementation of nets, such as Carifend®, can serve as a straightforward yet highly effective deterrent against the infestation of stored products by these pests.

To the best of our knowledge, this is the first research that evaluated the effectiveness of Carifend® against larvae. Nonetheless, other research studies have

explored the effectiveness of various insecticidal nets against the larvae of certain species within this group (Agrafioti et al., 2020; Wilkins et al., 2020). For example, Agrafioti et al. (2020) evaluated the insecticidal properties of four net formulations coated with SiO_2 nanoparticles, against *T. confusum* larvae, individuals of the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) and *S. oryzae* adults. Among the species tested, larvae of *T. confusum* exhibited the lowest mortality rate, emphasizing the significance of controlling larvae of this species. In addition, Wilkins et al. (2021) assessed the efficacy of Long-Lasting Interpreted Nets against immature *T. castaneum* and T. *variabile* in comparison to adult specimens. The movement and dispersal ability of the insects were examined following exposure to either LLIN or an untreated control netting. Interestingly, the larval stage of each species exhibited higher tolerance to the insecticidal netting compared to the adult stage. Furthermore, several previous studies have consistently reported that larvae, particularly those in larger developmental stages, exhibit greater tolerance to insecticides than their adult counterparts (Athanassiou et al., 2015; Saglam et al., 2013). Notably, large larvae are deemed more tolerant due to their proximity to the pre-pupation period, a phase that significantly influences factors such as mobility, metabolism, and behavior. These physiological aspects, as elucidated by Cox et al. (1984), contribute to their heightened resilience to insecticides. Consequently, the strategic application of insecticides, especially when a substantial portion of the population is in the late-instar larval stage, may prove to be less effective in achieving desired control outcomes. This emphasizes the need for deeper understanding of the developmental stage-dependent susceptibility of insect populations to optimize the efficacy of pest management strategies.

On the other hand, many studies have focused on investigating the sublethal effects of this method (Morrison et al., 2018; Wilkins, et al., 2020; Domingue et al., 2021;

Gerken et al., 2021). According to Rumbos et al., (2018), although immediate mortality rates were found to be low for both *L. serricorne* and *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), even after a 24-hour exposure period, a significant level of immediate knockdown was observed, particularly when the exposure duration exceeded 60 minutes. Subsequently, following the removal of insects from the dishes that were treated with Carifend®, the initially high knockdown percentages gradually transitioned, in most cases, into delayed mortality (Rumbos et al., 2018). In addition, Agrafioti et al. (2023) conducted an examination of the efficacy of Carifend® against a group of stored product beetles. Their findings revealed that, despite the variation in the initial exposure interval's effect on delayed mortality, the majority of cases exhibited relatively comparable delayed effects. This suggests that even a 2-hour exposure period was sufficient to induce a significant level of mortality, underscoring the importance of evaluating the delayed effects of treatments. The significance of delayed mortality has been emphasized in studies involving several pyrethroids across various stored-product insect species (Arthur, 1998, 1999; Agrafioti et al., 2015; Agrafioti et al., 2023). These results highlight the broader implications for understanding and considering the delayed effects of treatments in pest management strategies for stored product insects.

5. Conclusion

In conclusion, this research is the first to present data from laboratory bioassays regarding the impact of Carifend® on the larvae of five important stored product pests. This study also underscores the need for further investigation into the sublethal effects and the developmental stage-dependent susceptibility of insect populations to refine and optimize pest management strategies. The integration of both physical and chemical elements in pest control measures, as demonstrated by Carifend®, presents a promising avenue for comprehensive and sustainable stored product insect management. Further research and refinement of these strategies are essential to advance our understanding and enhance the efficacy of pest control interventions in stored product ecosystems. We suggest that this technology has the potential for adoption across various facilities, including its use in food packaging, where the larval stage can easily penetrate.

CONCLUSIONES

CONCLUSIONES/ CONCLUSIONS

1. El estudio de daños e infestación en higos secos en función del nivel de manejo y régimen hídrico del cultivo ha puesto de manifiesto que, en las plantaciones de regadío, las labores agrícolas son fundamentales para reducir significativamente la incidencia de agentes bióticos que degradan su calidad durante el desarrollo y secado del higo.

The study of damage and infestation in dried figs as a function of the level of management and water regime of the crop has shown that, in irrigated plantations, agricultural labor is essential to significantly reduce the incidence of biotic agents that degrade the quality during the development and drying of the fig.

2. En Extremadura, las aves fueron la principal causa de daños en higos secos, seguidas de insectos y mohos. Las plagas de insectos predominantes fueron *Cadra figulilella*, *Carpophilus hemipterus* y *Ceratitis capitata*. En cuanto a mohos, el género *Aspergillus* prevaleció en todas las plantaciones estudiadas, seguido de *Alternaria* en las establecidas en condiciones de regadío.

In Extremadura, birds were the leading cause of damage to dried figs, followed by insects and moulds. The predominant insect pests were *Cadra figulilella*, *Carpophilus hemipterus* and *Ceratitis capitata*. As for moulds, the genus *Aspergillus* prevailed in all the plantations studied, followed by *Alternaria* in those established under irrigated conditions.

3. El sistema de mallas Witty adaptado a la copa de la higuera redujo considerablemente los higos secos dañados por aves e infestados por insectos del orden Lepidóptera. Además, este sistema disminuyó significativamente la incidencia de la mayoría de las micotoxinas detectadas.

The Witty netting system adapted to the fig tree canopy significantly reduced dried figs damaged by birds and infested by insects of the order Lepidoptera. In addition, this system significantly decreased the incidence of most of the mycotoxins detected.

4. Los higos secos de la variedad Calabacita mostraron mejores características fisicoquímicas (peso del fruto, color y firmeza) cuando la maduración y el secado tuvo lugar con el sistema de mallas. Además, la malla suspendida para el secado y recolección de los higos evitó su contacto con el suelo, lo que propició la producción de frutos de alta calidad higiénico-sanitaria y facilitó su recolección.

Dried figs of the Calabacita variety showed better physicochemical characteristics (fruit weight, colour and firmness) when ripening and drying occurred with the net system. In addition, the suspended netting for drying and harvesting the figs prevented contact with the ground, favouring the production of high hygienic-sanitary quality fruits and facilitating their harvesting.

5. La congelación mostró ser una alternativa eficaz en el control de las plagas de insectos del higo seco en la industria, contribuyendo a reducir el uso de productos químicos sintéticos como el fosfuro de aluminio. Los tratamientos de 1 día a -18ºC, 2 días a -10ºC y 7 días a -5ºC, fueron los óptimos para alcanzar el 100% de la mortalidad de las plagas.

The freezing treatments proved to be an effective alternative in controlling insect pests of dried figs in the industry, contributing to reducing the use of synthetic chemical products such as aluminium phosphide. Treatments of 1 day at -18ºC, 2 days at -10ºC and 7 days at -5ºC were optimal for achieving 100% pest mortality.

6. En industria, el tratamiento de congelación de los higos secos de la variedad Calabacita durante 7 días a -5ºC influyó positivamente en sus características sensoriales, mientras que el tratamiento de 2 días a -10ºC preservaba mejor las características bioactivas.

In industry, the freezing treatment of dried figs of the Calabacita variety for 7 days at -5°C positively influenced their sensory characteristics, while the 2 days treatment at -10°C better preserved the bioactive characteristics.

7. El uso de la malla impregnada en alfacipermetrina mostró una buena eficacia en el control físico y químico de larvas de las plagas de almacén *Tribolium confusum*, T*ribolium castaneum, Alphitobius diaperinus, Tenebrio molitor* (Coleoptera: Tenebrionidae) y *Ephestia kuehniella* (Lepidoptera: Pyralidae). El uso de este tipo de sistema durante el almacenamiento podría ser una estrategia adecuada para evitar la reinfestación de los higos secos en esta etapa.

The use of alfa cypermethrin-impregnated netting showed good efficacy in the physical and chemical control of larvae of the storage pests *Tribolium confusum*, T*ribolium castaneum, Alphitobius diaperinus, Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae). The use of this type of system during storage could be a suitable strategy to avoid reinfestation of dried figs at this stage.

8. La implementación de las diversas estrategias de control de plagas en el higo seco a lo largo de su proceso productivo evaluadas en esta Tesis Doctoral, como prácticas agronómicas de manejo del cultivo en campo, el uso de un sistema de malla durante el secado en el árbol, la congelación en la industria y uso de mallas impregnadas con alfacipermetrina durante el almacenamiento, permitiría mejorar significativamente la

calidad higiénico-sanitaria del producto final. Asimismo, podría reducir las pérdidas ocasionadas por daños, impulsar la comercialización del higo seco y fomentar la sostenibilidad del cultivo de la higuera.

The implementation of various pest control strategies in dried figs throughout the production process evaluated in this Doctoral Thesis, such as agronomic practices for crop management in the field, the use of netting system during drying on the tree, freezing in the industry and the use of netting impregnated with alfa cypermethrin during storage, would significantly improve the hygienic-sanitary quality of the final product. It could also reduce losses due to damage, boost the commercialization of dried figs and promote the sustainability of fig cultivation.

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ANEXO

ANEXO: PRODUCCIÓN CIENTÍFICA

En la Tabla 1 se muestran los principales méritos científicos obtenidos durante la etapa predoctoral en campo temático de la tesis doctoral.

Tabla 1. Producción científica: artículos indexados en JCR, capítulos de libro, artículos de divulgación y comunicaciones a congresos.

	Título trabajo	Tipo	Estado	Anexo
Publicaciones científicas de la tesis	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).	Atículo científico	Publicado Revista Crop Protection Q2 (Agronomy)	I
	Implementation of Witty® net system for production of 'Calabacita' dried figs: effects on pest incidence, fruit quality and mycotoxin occurrence.	Artículo científico	Revisión por los autores	-
	Freezing treatments as an alternative to conventional pest control in dried figs and their effect on global fruit quality.	Artículo científico	En revisión - Journal of Stored Products Research Q1	-
	Evaluation of Carifend® for the control of larvae of major stored-product species.	Artículo científico	Revisión por los autores	-
	Análisis del Sistema de formación en espaldera en el cultivo de la higuera.	Artículo revista divulgación	Publicado Vida Rural, 505	II

	Título del trabajo	Tipo	Congreso	Anexo
Congresos nacionales o jornadas	Evaluación de daños en recolección de higos secos cultivados bajo condiciones de superintensivo en Extremadura.	Comunicación Poster	Congreso En Red De Olivicultura, Citricultura Y Fruticultura De La Sociedad Española De Ciencias Hortícolas (Online). Marzo 2021	III
	Evaluación agronómica y de calidad de fruto de las variedades San Antonio y Dalmatie cultivadas en espaldera.	Comunicación Oral		IV
	La congelación como alternativa potencial a la aplicación de fosfuros para el control de insectos durante el almacenamiento del higo seco	Comunicación Poster – (Premio mejor póster).	XVI Congreso Nacional Ciencias Hortícolas (Córdoba). Octubre-2021.	V
	Evaluación de daños e identificación de plagas y hongos del higo seco en las principales zonas productoras de Extremadura.	Comunicación oral		VI
	"Control bìológico de parásitos de la higuera".	Comunicación Oral.	Jornadas De Proyectos De Investigación Para La Sostenibilidad De Las Producciones Agrícolas Y Ganaderas (Economía Verde Y Circular). Mérida. Diciembre 2019	VII
Congresos Internacionales	Evaluación de la aptitud al secado de las variedades de higuera `Zidi' y `Conadria'. (Póster científico)	Comunicación Poster	XIII Congreso Nacional Y XI Ibérico De Maduración Y Postcosecha (Zaragoza) Junio 2022	VIII
	Evaluación del sistema de mallas Witty® para el secado del higo y su influencia en la calidad físico-química.	Comunicación Oral	IX Congreso Ibérico Y XVII Congreso Nacional De Ciencias Hortícolas (Mérida). Junio 2023.	IX
	Freezing as potential alternative to the application of phosphides for the control of insects during the storage of dried figs	Comunicación Poster	Workshop: Valorizing the diversity of the fig tree, an ancient fruit crop for sustainable mediterranean agriculture: the prima project "FIGGEN". (Badajoz) Julio 2023.	X

	Titulo	Tipo	Detalles	Anexo
Colaboraciones científicas	Orchard Establishment and Management.	Capítulo libro	Libro: The fig: botany, production and uses. (pp. 184–230). GB: CABI. ISBN: 10.1079/9781789242881.0008	XI
	Implementation of super high-density systems and suspended harvesting meshes for dried fig production: Effects on agronomic behaviour and fruit quality	Artículo científico	Revista Scientia Horticulturae Q1 (Horticulture)	XII
	Sistema de producción de higos secos en superintensivo, una alternativa rentable.	Artículo divulgación	Revista Vida Rural, 472.	XIII

ANEXO I

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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; Weiss, 2015). The world fig production was 1.34 million tons in 2021 (FAOSTAT, 2021), 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, 2021). Fresh figs are extremely perishable, and drying them has been the most widespread way to preserve them for a longer period (Veberic et al., 2008), thus facilitating their transport, storage and availability. Today, most commercially produced figs are dried or processed (Flaishman et al., 2008; Shokoohi et al., 2022).

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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ANEXO II

Análisis del sistema de formación en espaldera en el cultivo de la higuera

Se evalúan los distintos aspectos agronómicos y de calidad de frutos

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En las últimas décadas la necesidad de los fruticultores de las Vegas del Guadiana por la diversificación de los cultivos ha favorecido la introducción de nuevas especies o la implantación de otros cultivos tradicionales de secano a sistemas de producción en intensivo con riego localizado. Esta necesidad, junto con el incremento de la demanda de higos frescos y secos en los mercados nacionales e internacionales, ha propiciado que la higuera se haya posicionado como uno de los cultivos alternativos en Extremadura.

Detalle de Colar Elche cultivada en espaldera.

El sector frutícola en Extremadura representa una parte importante de la producción agrícola total, con una superficie de 49.510 ha dedicadas en 2020 y un potencial productivo de unas 320.000 t (MAPA, 2020). La producción nacional de higo ocupa el sexto lugar en el ranking mundial, siendo líder entre los países de la Unión Europea, con un total de 59.900 t en el año 2020. Dentro de España, Extremadura se sitúa como el principal productor con un 62,4% de la producción nacional y, durante la última década, ha visto incrementada su superficie de cultivo, pasando de 5.057 a 7.034 ha y su producción de 10.384 t a 37.382 t (MAPA, 2020).

El principal destino de esta producción es para el consumo en seco, al contrario que ocurre en otras zonas productoras españolas como la Comunidad Valenciana o Cataluña, cuya producción se centra en el consumo en fresco tanto de brevas como de higos. La demanda del producto en fresco va en aumento y, según un estudio realizado por Future Market Insights (FMI) se prevé una tasa anual de crecimiento del 5,3% entre 2018 y 2027. Estas previsiones de futuro junto con los precios percibidos por los fruticultores han propiciado el incremento de nuevas plantaciones destinadas a la producción en fresco. Ge-

ANEXO III

Evaluación de daños en recolección de higos secos cultivados bajo condiciones de superintensivo en Extremadura.

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Extremadura es la principal Comunidad Autónoma en superficie y producción de higuera, cuyo destino principal es el consumo en seco. Tradicionalmente se ha cultivado en secano, con amplios marcos de plantación y cuyos higos secos son recogidos del suelo a medida que avanza su proceso de senescencia en el árbol. Posteriormente, estos higos son recolectados durante el verano, en 2 ó 3 pasadas, con porcentajes muy variables de pérdidas en la producción por la incidencia de diferentes tipos de daños, así como de plagas y enfermedades que afectan a su calidad higiénico-sanitaria. En el año 2010, el Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX) estableció en regadío un ensayo de higuera en superintensivo para la producción de higo seco como consecuencia del incremento de su demanda, junto con la necesidad del sector frutícola de introducir nuevos cultivos con potencial productivo en las zonas regables. Además, para mejorar y facilitar el proceso de recolección de los higos secos, en este ensayo se emplearon unas estructuras para colocar redes a unos 50 cm del suelo. El objetivo de este trabajo fue evaluar los daños físicos y la presencia de parásitos en higos secos recolectados en redes procedentes de tres variedades cultivadas en superintensivo. En cuanto a los resultados obtenidos en 2019, los porcentajes de higos dañados en el total de la producción fueron mayores en la variedad 'Cuello Dama Blanco' con un 47,6%, seguido de la variedad 'Picholetera' y 'Calabacita' con 38,1% y 27,5%, respectivamente. La principal causa de daños en los higos de las tres variedades fue las picaduras de pájaros, que oscilaron sobre el total de daños entre un 53% y 56%, seguidos de ennegrecimiento parcial o completo del fruto (entre un 21% y 29%, dependiendo de la variedad). En cuanto a la presencia de parásitos en el interior de los higos dañados, el porcentaje osciló entre un 9,5% y 28,8%, predominando el parasitismo por hongos, coleópteros y dípteros de la especie *Ceratitis capitata*. La identificación y cuantificación de estos daños a lo largo de la campaña permitirá implantar medidas eficaces de control, así como una gestión integrada de plagas, que permitirán controlar y/o disminuir el riesgo de contaminación y obtener higos de elevada calidad higiénico-sanitaria.

Palabras claves: *Ficus carica* L., plagas, parásitos, higos secos

ANEXO IV

Evaluación agronómica y de calidad de fruto de las variedades San Antonio y Dalmatie cultivadas en espaldera.

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El cultivo de la higuera (*Ficus carica* L.) con destino al consumo en fresco está adquiriendo gran importancia en las últimas décadas. La preocupación de la población por comer alimentos más saludables ha provocado un aumento de la demanda en mercados nacionales e internacionales. Los higos frescos son recolectados manualmente de árboles formados generalmente en vaso con marcos amplios y con riegos localizados. El color en la piel de los higos generalmente varía de negro a morado y a tonos más claros como el verde o amarillo. En el año 2016, el Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX), se establecieron dos variedades potencialmente comerciales de distinta tonalidad de piel, 'San Antonio' y 'Dalmatie', en intensivo (4 x3 m) y formadas en espaldera. Este tipo de formación favorece la aireación, soleado y por ende la maduración homogénea de los frutos, facilitando además su recolección. El objetivo de este trabajo fue evaluar la aptitud agronómica de estas variedades y la calidad de los higos recolectados en este sistema de formación desde su entrada en producción en 2018 hasta 2020. En cuanto a los resultados obtenidos, la variedad 'Dalmatie' fue la más productiva con 28,8 kg/árbol en el año 2020, a diferencia de 'San Antonio' que obtuvo 16,8 kg/árbol ese mismo año. El calibre y peso medio del fruto obtenido fue de 42,6 mm y 43,3 g para San Antonio, superada por 'Dalmatie' con 48,4 mm y 71,8 g. `San Antonio' presentó mayor contenido total de sólidos solubles con 20,8 ºBrix frente a 19,2 ºBrix de 'Dalmatie'. Los valores de acidez titulable fueron similares, con un valor medio de 0,11 en 'San Antonio' y de 0,14 en 'Dalmatie'. El pH medio de ambas variedades osciló entre 5,34 -5,92. Como conclusión, ambas variedades presentan alto potencial productivo y de calidad de fruto en este sistema de formación, destacando la variedad Dalmatie por la alta producción y el elevado calibre y peso medio de los higos.

Palabras claves: *Ficus carica* L., higo fresco, espaldera, calidad.

ANEXO V

XVI Córdoba 2020 Congreso Nacional Ciencias Hortícolas

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La congelación como alternativa potencial a la aplicación de fosfuros para el control de insectos durante el almacenamiento del higo seco

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Palabras Clave:
Ficus carica L., plagas de almacén, insectos, fosfina, tratamiento

RESUMEN:
El consumo de higo seco ha experimentado un crecimiento significativo en los últimos años. Una sociedad con hábitos de vida cada vez más saludables, junto a un producto rico en nutrientes y beneficioso para la salud, ha promovido que el higo seco adquiera una mayor importancia. La deshidratación de los frutos se lleva a cabo de forma natural en el árbol hasta que caen al suelo, donde terminan su secado al sol. Durante este periodo, los higos pueden ser afectados por diferentes especies de insectos, pudiendo comprometer su calidad higiénico-sanitaria. Para evitar la aparición de dichos insectos durante el almacenamiento y comercialización del higo seco, las industrias aplican un curado con fosfuro de aluminio y/o magnesio (i.e., fosfina), antes del procesado del producto. Este tratamiento está cada vez más en desuso, por las limitaciones legislativas, así como por el impacto negativo del uso de productos químicos. Nuestro objetivo fue evaluar la eficacia de diferentes tratamientos de congelación como alternativa a la "fosfina". Los tratamientos fueron de 48 horas con temperaturas de -5ºC y -18ºC. Los frutos tratados tenían una infestación media de 44,6%, provocada de forma natural por insectos de las especies *Cadra abstersella*, *Plodia interpunctella* (Lepidoptera) y *Carpophilus hemipterus* (Coleoptera). Los resultados muestran que la temperatura más efectiva fue -18ºC, causando una mortalidad del 100% de los insectos carpófagos mencionados. El tratamiento a -5ºC sólo provocó una mortalidad del 55% en los lepidópteros y del 90% en los coleópteros. Los resultados sugieren que la congelación (-18ºC durante 48 h) es una alternativa eficaz para el control de las plagas de almacén del higo seco, reduciendo los tiempos de tratamiento en postcosecha y sobre todo evitando el uso de productos químicos.

ANEXO VI

XVI Córdoba 2020 Congreso Nacional Ciencias Hortícolas

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Evaluación de daños e identificación de plagas y hongos del higo seco en las principales zonas productoras de Extremadura.

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Palabras Clave:
Ficus carica L., plagas de almacén, insectos, hongos, control integrado, pérdidas.

RESUMEN:
Extremadura ha multiplicado 3,6 veces la producción total de higos entre las campañas 2010 y 2020, siendo el consumo en seco su principal destino. En agosto de 2020 durante la campaña de recolección de higo seco, el Centro de Investigaciones Científicas y Tecnológicas de Extremadura llevó a cabo un muestreo de higos secos en ocho higuerales representativos de las diferentes zonas productoras de la región. Nuestro objetivo fue evaluar los daños en fruto y determinar las especies de plagas y hongos causantes de daños mediante técnicas de identificación morfológica y biología molecular. Las muestras se clasificaron según el origen del daño: insectos, hongos, pájaros, aguados y otros. Los resultados mostraron una alta variabilidad dependiendo de la localización. El porcentaje de frutos que presentaron algún tipo de daño o alteración osciló en entre un 8,4 y un 47,1%. El principal daño registrado fue por picadura de pájaros en campo, con un porcentaje medio en todas las zonas muestreadas de 12,0%, seguido del causado por insectos 7,4%. Entre las especies carpófagas predominaron *Carpophilus hemipterus* (Coleoptera), *Cadra figulilella* y *Ectomyelois ceratoniae* (Lepidoptera) y *Ceratitis capitata* (Diptera). Sólo un 0,4% de los higos mostraron crecimiento visible de hongos en su interior, siendo prevalente el género *Aspergillus*. La cuantificación de los daños y la identificación de sus agentes causales, permite implementar sistemas de control y manejo más eficaces en la producción de higo seco, obteniendo frutos de mayor calidad higiénico-sanitaria y reduciendo el porcentaje de pérdidas.

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CERTIFICADO PARTICIPACIÓN

D. Antonio Jesús Galán Jiménez, con DNI: 80091590R, ha participado en las Jornadas "PROYECTOS DE INVESTIGACIÓN PARA LA SOSTENIBILIDAD DE LAS PRODUCCIONES AGRÍCOLAS Y GANADERAS (ECONOMÍA VERDE Y CIRCULAR)", con la ponencia ***"Control biológico de parásitos en higuera"***

Actividad de transferencia de los Proyectos Estratégicos Sectoriales de CICYTEX. (Resolución del 14 de diciembre de 2016, por la directora de CICYTEX, periodo 2016-2019)

Organizadas por el grupo de Transferencia de CICYTEX, el 4 de diciembre de 2019, en el Palacio de Congresos de Mérida.

Y para que conste, expido y firmo el presente certificado en Guadajira, a 20 de enero de 2020.

Carmen González Ramos

Directora del Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX)

ANEXO VIII

Evaluación de la aptitud al secado de las variedades de higuera 'Zidi' y 'Conadria'

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Resumen

El higo seco es uno de los principales frutos secos consumidos en todo el mundo. Este producto se caracteriza por ser una buena fuente de vitaminas, minerales, carbohidratos, fibra y compuestos fenólicos. Extremadura lidera la producción de higo en España, cuyo destino principal es la producción de higo seco. Por tradición, las variedades cultivadas para la producción en seco en esta región son 'Calabacita', 'Picholetera', 'La Casta' y 'Cuello Dama Blanco', todas ellas con frutos de color de piel entre el amarillo verdoso y el verde amarillento. Pese a ello, cada vez son más los productores que muestran interés en la búsqueda de variedades que les permitan acceder a nuevos mercados. El objetivo de este trabajo ha sido evaluar la aptitud al secado en términos de calidad de dos variedades de higuera de diferente tonalidad de piel, 'Zidi' morada y 'Conadria' verde amarillento. Para ello, durante la campaña de 2021, se recolectaron higos frescos de estas variedades, realizándose el proceso de deshidratado de forma natural en un secadero solar y, posteriormente, se evaluó su calidad. En cuanto a los parámetros de calidad, se determinó peso, firmeza, color, contenido en sólidos solubles (CSS), acidez titulable, pH, humedad y actividad de agua (a_w), al inicio y al final del secado. Durante este periodo, la variedad 'Zidi' tuvo una pérdida de peso del 76,8% y 'Conadria' de 60,5%, con un peso medio final de 20,7 y 10,9 g, respectivamente. El CSS final fue de 82,6 °Brix para 'Conadria' y de 80,6 °Brix para 'Zidi'. La firmeza aumentó tras el secado con valores medios de 1,5 y 2,1 N/mm para 'Zidi' y 'Conadria', respectivamente. El grado de aceptación sensorial de ambas variedades fue bueno, obteniéndose una puntuación media de 5,8 para 'Conadria' y 7,3 para 'Zidi' sobre 10. En conclusión, estos resultados sugieren que las variedades estudiadas mostraron una calidad adecuada y podrían, junto con las variedades tradicionales, ampliar la oferta comercial en base a las necesidades de los mercados.

Palabras clave: *Ficus carica* L., higo seco, calidad, actividad de agua, sensorial

S4-O5

Evaluación de un sistema de mallas adaptado a la higuera para el secado de los higos y su influencia en la calidad físico-química del higo

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Palabras clave: *Ficus carica* L., control, daños pájaros, insectos, mohos

En las últimas décadas, el incremento de la demanda de higo seco ha propiciado la profesionalización del sector, incorporando nuevas técnicas de cultivo como el riego y los marcos de plantación más intensivos. Sin embargo, el proceso de secado y recolección continúa realizándose de forma tradicional. El higo maduro comienza a deshidratarse en el árbol, más tarde cae al suelo dónde finaliza su secado. Durante este transcurso los frutos pueden deteriorarse por diversos agentes bióticos como son pájaros, insectos y hongos. La empresa W Lagro Consultores ha diseñado un sistema de mallas (Witty®) adaptado a la copa del árbol para proteger los higos a lo largo del proceso de secado y recolección contra los principales agentes externos. El objetivo de este trabajo fue evaluar la eficacia de este sistema frente al sistema tradicional en cuanto a los agentes bióticos y la calidad fisicoquímica de los higos secos. Los resultados mostraron un descenso significativo de pérdidas por daños de pájaros medio de 19,4% y una reducción en un 4% de higos secos parasitados por insectos de este sistema frente al tradicional. Los pesos medios de los frutos fueron 10,1 y 11,4 g, con humedades medias de 22,3% y 23,5%, para el secado en suelo y con la malla respectivamente. En cuanto a los sólidos solubles, firmeza y actividad de agua no se encontraron diferencias significativas. Este sistema puede ser una alternativa viable para reducir daños y por consecuente pérdidas económicas producidas durante la exposición del higo en el árbol, sin llegar a comprometer su calidad físico-química.

ANEXO X

WORKSHOP

VALORISING THE DIVERSITY OF THE FIG TREE, AN ANCIENT FRUIT CROP FOR SUSTAINABLE MEDITERRANEAN AGRICULTURE: THE PRIMA PROJECT "FIGGEN"

05TH. JULY 2023

Auditorium of INTAEX (Avenida Adolfo Suárez s/n) Badajoz (SPAIN)

CERTIFICATE OF PRESENTATION

AWARDED TO

Dr. Antonio Jesús Galán

Presenter of the poster

"Freezing as potential alternative to the application of phosphides for the control of insects during the storage of dried figs"

Coordinator of project FIGGEN - Prof. Tommaso Giordani

University of Pisa · CICYTEX Centro de Investigaciones Científicas y Tecnológicas de Extremadura · CSIC Consejo Superior de Investigaciones Científicas · Cukurova University · Faculté des Sciences de Tunis

ANEXO XI

8 Orchard Establishment and Management

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8.1 Introduction

Traditionally fig was cultivated under rainfed conditions at low densities and the fruit processed primarily into dried figs. Modern fig orchards are now irrigated and planted at moderate to high densities to produce both fresh and dried figs. This chapter summarizes how fig growers can take advantage of the wide range of edible fig cultivars, their collective climatic and edaphic adaptability and the multiple methods of manipulating fig production to achieve the global fig production of today.

8.2 Site Selection

Prior to establishing a modern fig plantation, microclimate, irrigation water availability and quality, and the soil physical and chemical characteristics should be assessed. These topics are covered in detail in Chapter 7: *Environmental Requirements and Site Selection*, which summarizes site selection as follows 'Although the cultivated fig (*Ficus carica* L.) is a subtropical climate plant, it is grown successfully in many areas of the world that have cool winters and hot dry summers.' Fig trees are especially well adapted to Mediterranean climate conditions. However,

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ANEXO XII

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Implementation of super high-density systems and suspended harvesting meshes for dried fig production: Effects on agronomic behaviour and fruit quality

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ABSTRACT

The demand for dried figs has increased in recent years. This, together with rising production costs and difficult access to labour for harvesting, has made it necessary to seek alternatives to the traditional systems. The fig tree is a fruit species with a high adaptive capacity. In this work, we studied for 5 consecutive years the adaptation of the Calabacita variety, traditionally used for dried fig production in the southwest of Spain, under high density conditions (1000 trees per hectare with a planting frame of 5 × 2 m). In addition, a suspended mesh netting structure was installed to facilitate the harvesting process. Compared to the traditional system, the results obtained with this system are higher. In addition to improving the harvesting operation, use of the suspended mesh netting may also reduce damage caused by insects. The dried figs obtained were firmer and had a darker brown colour than those using the traditional system, probably because the suspended meshes favour the drying process by facilitating air circulation. Finally, use of the suspended mesh netting also constitutes an important advance from a sanitary perspective, as the lower moisture content of the figs harvested this way impedes the proliferation of mycotoxigenic fungi and, consequently, the possible presence of mycotoxins.

1. Introduction

In traditional production systems, the fig tree has always been cultivated in low-density orchards (less than 100 trees/ha), characterised by very limited management and irrigation, and with generally low yields (López-Corrales et al., 2012). In modern fruit orchards, to increase profitability, new high-density formation systems have begun to be introduced using dwarfing rootstock and training systems that ensure maximum sunlight interception to guarantee higher fruit quality and yield, as well as better adaptation of these systems to facilitate operations such as harvesting, pruning, etc. (Iglesias, 2019). Thus, nowadays, in other fruit species such as olive, apple or citrus, intensive and super-intensive density systems have acquired a high degree of popularity (Iglesias, 2019). Previous studies on olive trees, for example, have shown that yield and fruit quality are higher with high-density plantations and irrigation systems (Ahumada Orellana et al., 2018; Martorana et al., 2017). A study carried out by Milosevic et al. (2008) showed greater precocity and higher yield and return on investment in super-intensive plum cultivation compared to the traditional system. In similar traditional crops such as almond, irrigation is considered the main limiting factor of yield in terms of number of fruits and quality (Gutiérrez-Gordillo et al., 2019).

Fig cultivation is strongly affected by climatic conditions. For this reason, 70 % of the world's fig production is concentrated in countries on the Mediterranean coast (Arpaci, 2017), with Turkey the leading producer with 305.698 t. Spain is Europe's leading producer with 47.750 t. Extremadura, in the southwest of Spain, has the country's largest cultivated area (6.104 ha), mostly for the production of dried figs, although figs can also be also consumed fresh, processed or in fig paste (Vebric et al., 2008). In this area, only 14 % of the area is

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ANEXO XIII

Sistema de producción de higos secos en superintensivo, una alternativa rentable

El cultivo se realiza en regadío y con recolección mediante mallas

La implantación de empresas agroalimentarias de Extremadura y Castilla y León basadas en la venta de higos secos y sus transformados ha propiciado que el sector productivo empiece a considerar a la higuera como una alternativa real. Desde el Centro de Investigaciones Científicas y Tecnológicas de Extremadura (Cicytex) se planteó la necesidad de implementar nuevas técnicas de cultivo en regadío para la producción de higos para consumo en seco unido al desarrollo de un sistema de recolección más eficiente y que mantuviese los higos con la máxima calidad organoléptica y sanitaria.

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La higuera (*Ficus carica* L.) es un cultivo característico del área mediterránea que se distingue de otros frutales principalmente por la producción de dos cosechas (brevas e higos), que pueden ser consumidos tanto en fresco como en seco. La superficie mundial se cifra en torno a las 315.500 ha, con una producción superior al 1.100.000 t anuales siendo Turquía el principal país productor, con el 23% de dicha producción mundial. En Europa, España es el principal país productor con unas 13.979 ha y una producción total de 47.742 toneladas, que suponen el 35% de la producción europea y un 3,5% de la producción mundial (Faostat, 2017). De esta superficie, más del 85% se cultiva en secano, liderando Extremadura la superficie y la producción con unas 5.824 ha y unas 18.755 t cuyo destino principal es la producción de higos para el consumo en seco.

A pesar de ser un cultivo tradicional, ampliamente distribuido y característico de la producción agrícola en la Península Ibérica desde hace milenios, es una especie poco estudiada tanto en técnicas de cultivo como en material vegetal. En rela-