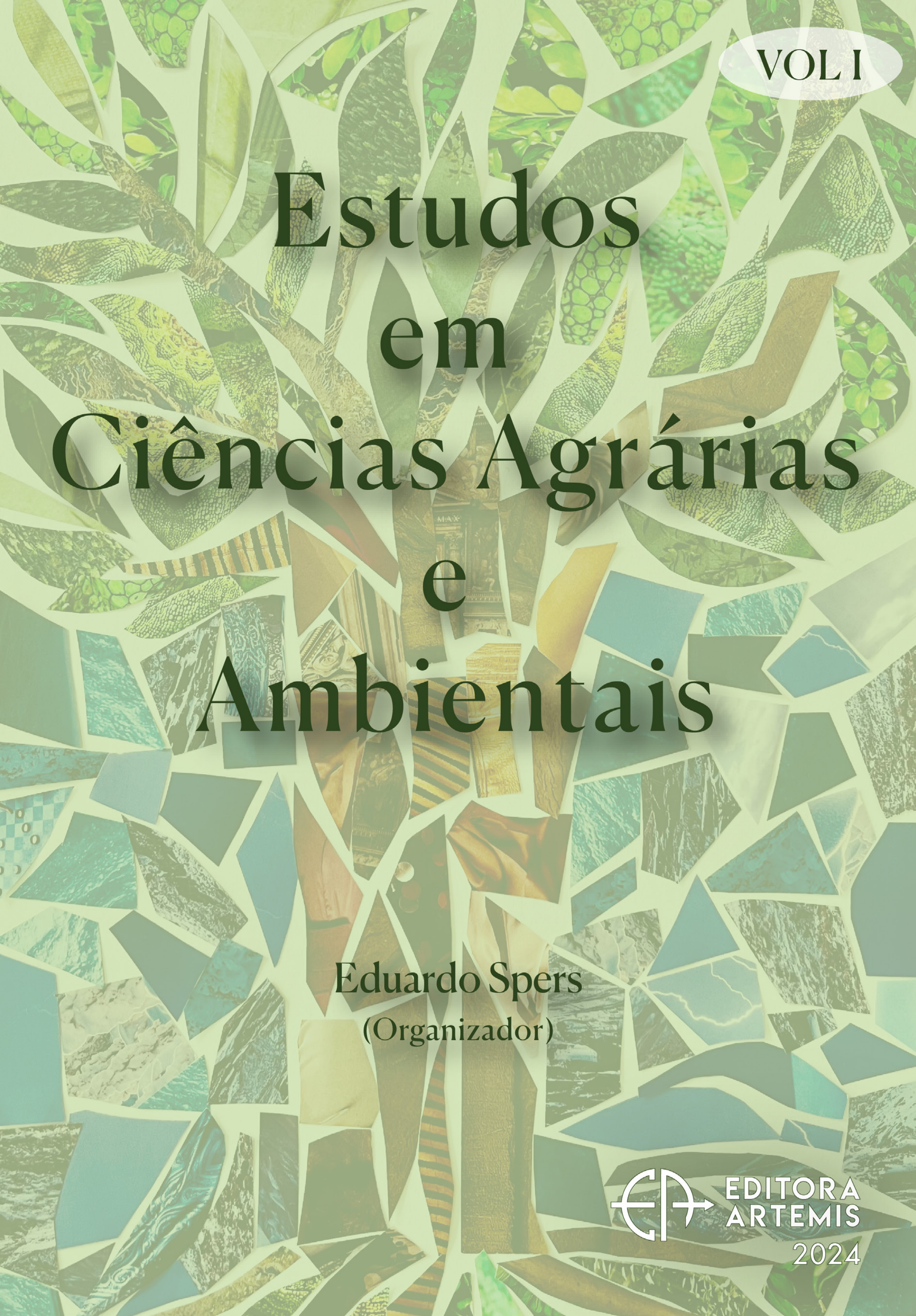

VOL I

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

EDITORA ARTEMIS
2024

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Bibliotecário	Maurício Amormino Júnior – CRB6/2422

EDITORA ARTEMIS
2024

Editora Artemis
Curitiba-PR Brasil
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e-mail:publicar@editoraartemis.com.br

EDITORA ARTEMIS
2024

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

E82 Estudos em Ciências Agrárias e Ambientais I [livro eletrônico] / Organizador Eduardo Eugênio Spers. – Curitiba, PR: Artemis, 2024.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
Edição bilíngue
ISBN 978-65-81701-19-2
DOI 10.37572/EdArt_300724192

1. Ciências agrárias – Pesquisa – Brasil. 2. Meio ambiente. 3.Sustentabilidade. I. Spers, Eduardo Eugênio.

CDD 630

Elaborado por Maurício Amormino Júnior – CRB6/2422

EDITORA ARTEMIS
2024

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

APRESENTAÇÃO

O campo das ciências agrárias e ambientais está em constante evolução, refletindo a necessidade crescente de entender e gerenciar os recursos naturais e a produção agrícola de maneira sustentável.

O primeiro volume desta nova coletânea "**Estudos em Ciências Agrárias e Ambientais"**, reúne 12 capítulos de destacados pesquisadores, oferece uma visão abrangente das investigações mais recentes em quatro eixos cruciais e complementares: ciências agrárias, ciências dos animais, ciências dos alimentos e ciências ambientais.

No eixo **Estudos em Ciências Agrárias**, os artigos exploram a variabilidade genética e os métodos de cultivo que podem influenciar a produtividade e a qualidade das culturas. O estudo da heterose em sementes híbridas de milho azul (cap. 1) revela como características superiores podem ser obtidas por meio de cruzamentos específicos. Adicionalmente, a análise do potencial genotécnico de híbridos e variedades sintéticas de milho azul (cap. 2) demonstra a importância da adaptação regional para maximizar a produtividade. A pesquisa sobre a manipulação de plantas de limão persa (cap. 3) e a propagação vegetativa do lúpulo (cap. 4) trazem insights sobre práticas de cultivo que podem otimizar a produção.

O eixo **Estudos em Ciências dos Animais** foca na saúde e na eficiência dos sistemas de produção animal. A detecção de imunoglobulinas contra *Anaplasma marginale* (cap. 5) é essencial para a compreensão das doenças bovinas, enquanto a avaliação da eficiência do uso de nutrientes em bovinos (cap. 6) pode melhorar a produtividade e a sustentabilidade das operações de pecuária. O estudo sobre a soroprevalência de *Mycobacterium avium* subespécie paratuberculosis em ovinos (cap. 7) oferece informações valiosas para o controle de doenças em sistemas de produção ovina.

Os artigos do terceiro eixo, **Estudos em Ciências dos Alimentos**, discutem a inovação e a funcionalidade na produção de alimentos. O potencial das sementes de *Moringa oleifera* (cap. 8) é explorado, destacando seus benefícios nutricionais e aplicações alimentares. Além disso, a dinâmica do status total de antioxidantes ao longo do processo de produção de vinho (cap. 9) revela como a qualidade do vinho pode ser monitorada e aprimorada, desde o suco até o produto final.

Finalmente, o eixo temático **Estudos em Ciências Ambientais** aborda questões cruciais relacionadas ao meio ambiente e à conservação. A investigação sobre a doença de manchas marrons e suas interações com hospedeiros (cap. 10) oferece uma visão sobre a gestão de doenças em agroecossistemas. Os avanços na conservação dos recursos genéticos de baunilha no México (cap. 11) são discutidos, evidenciando esforços para preservar espécies ameaçadas e a pesquisa sobre macrofauna bentônica em riachos (cap. 12) demonstra a importância dos organismos do solo para a saúde dos ecossistemas aquáticos.

Este livro não só apresenta pesquisas inovadoras e relevantes, mas também promove uma integração de conhecimentos que é vital para enfrentar os desafios contemporâneos nas ciências agrárias e ambientais. Acreditamos que as descobertas aqui compiladas contribuirão significativamente para o avanço da ciência e para a implementação de práticas mais sustentáveis e eficientes.

Desejo a todos uma proveitosa leitura!

Eduardo Eugênio Spers

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CAPÍTULO 10

INSIGHTS INTO BROWN SPOT DISEASE: CAUSAL AGENTS AND HOST INTERACTIONS IN AGROECOSYSTEMS

Data de submissão: 10/06/2024
Data de aceite: 21/06/2024

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ABSTRACT: The Brown Spot Disease is a ubiquitous fungal disease present in many vegetables and perennials. The mycotoxins produced by the disease lead to challenges in plant protection. Moreover, the actual fungicides are largely inadequate to deal with this disease aggressiveness. This aspect is a common feature of necrotrophic fungi. The studies on Brown Spot Disease were largely done by isolating the fungus from the infected tissues. Fungus belonging to *Stemphylium* sp. and *Alternaria* spp. were described on pear, apple, vegetables, and cover crops. The objective of this study was to obtain scientific evidence on the distribution of Brown Spot Disease over distinct plant species and clarify its infection mechanism. For that purpose, optical microscopy methods were used based on darkfield or polarized light, which build up significant steps for the comprehensive understanding of these fungus on their hosts.*Stemphylium vesicarium*, *Alternaria alternata* and *Alternaria arborescens* were associated to Brown Spot Disease symptoms. The fungus association and frequency depended on hosts and infected plant organs. In *Pyrus communis* cv. Rocha flowers *Alternaria* spp. were the most frequent. Later in the season, on maturation stage BBCH 85 *Stemphylium vesicarium* was the most frequent, particularly when the fruits turned yellow. It is likely that both *Stemphylium vesicarium* and *Alternaria* spp. act together to synergistically surpass the defence mechanisms of the hosts. The Brown Spot Disease pathosystem in Portugal is profusely diverse in pathogens and hosts.

KEYWORDS: *Stemphylium vesicarium*. *Alternaria alternata*. *Alternaria arborescens*. Light microscopy. Necrotrophic fungi.

ABORDAGEM SOBRE A DOENÇA DAS MANCHAS CASTANHAS: AGENTES CAUSAIS E INTERAÇÕES COM OS HOSPEDEIROS EM ECOSSISTEMAS AGRÁRIOS

RESUMO: A doença das manchas castanhas é uma doença ubíqua que causa danos em muitas espécies frutícolas e olerícolas. Pelo facto de a doença produzir micotoxinas, a sua proteção torna-se difícil e os fungicidas pouco eficazes. Este aspeto é uma característica de todas as doenças causadas por fungos necrotróficos. Os trabalhos realizados sobre a doença das manchas castanhas têm-se baseado em amostragem por isolamento dos tecidos infetados, demonstrando a presença e infecciosidade de fungos dos géneros Stemphylium e Alternaria em pereira, macieira, hortaliças e coberturas vegetais. O objetivo deste trabalho é produzir evidência científica sobre a distribuição dos fungos causadores da doença das manchas castanhas em diferentes hospedeiros e do seu mecanismo de infeção. Para este propósito foram implementadas técnicas de microscopia ótica baseadas na utilização de luz polarizada e de campo escuro, que permitem avanços significativos na compreensão da ação destes fungos nos seus hospedeiros. Foram identificados e documentados visualmente fungos das espécies *Stemphylium vesicarium*, *Alternaria alternata* e *Alternaria arborescens* associados aos sintomas da doença das manchas castanhas, em combinação e representatividade diferentes consoante os hospedeiros e órgãos infetados. Em *Pyrus communis* cv. Rocha o género Alternaria predomina no período da floração, enquanto na fase de maturação dos frutos a espécie *Stemphylium vesicarium* é a preponderante. É possível que estes fungos atuem de forma cooperativa para ultrapassar as defesas normais das plantas hospedeiras. O patossistema associado a esta doença é muito rico, pela diversidade de patogénios e hospedeiros, observados em várias zonas de Portugal.
PALAVRAS-CHAVE: *Stemphylium vesicarium*. *Alternaria alternata*. *Alteraria arborescens*. Microscopia ótica. Fungos necrotróficos.

1 INTRODUCTION

The Brown Spot Disease (BSD) is an important disease of pear worldwide. Reports published in Portugal (Francisco, 2021), The Netherlands (Köhl et al., 2013), Spain (Llorente and Montesinos, 2006), Italy (Köhl et al., 2009), France (Blancard et al., 1989), Poland (Hubert Głos et al., 2023), Argentina (Temperini et al., 2022) and Belgium (Van Laer et al., 2006) claim that *Stemphylium vesicarium* is the causal agent of BSD on pear. Nevertheless, *Alternaria* spp. can also cause symptoms compatible with those caused by *Stemphylium vesicarium* and this is often disregarded. The *Alternaria* spp. related with European pear hosts are *Alternaria alternata*, *Alternaria arborescens* and *Alternaria tenuissima*. Joyce Woudenberg (Woudenberg, 2015; Woudenberg et al., 2015) claimed that *A. tenuissima* is the same as *A. alternata*.

The aim of this study was to clarify the symptoms of both fungus and its relationship with different hosts, both perennial and annual species.

2 MATERIALS AND METHODS

From 2018 to 2020 two *Pyrus communis* cv. Rocha orchards were selected and winter buds, flowers, leaves and fruits were observed. From the two selected orchards, the one located in coordinates 39º19'5.82"N, 9º2'45.84"W (elevation 57 m) had historically low level of BSD (<3% on fruits) whereas the other, located at 39º19'40.20"N, 9º3'51.29"W (elevation 142 m) presented high levels of the disease (> 10% on fruits). Each year, 100 organs were selected: in wintertime (January and February) dormant flower buds were sampled, flowers in April, and from mid-June to mid-July leaves and fruits were collected and observed. Fruits were also observed in the week before harvest (mid-August).

In the high inoculum orchard, 50 fruits were sampled before and after spraying treatments in May and June (three times in total). The spray treatments were done with an air blast sprayer model Stagric 800 l (Stagric, Portugal).

Fruit samples were also collected on bell pepper (*Capsicum annum*), tomato (*Solanum lycopersicum*), fig trees (*Ficus carica*), cover crops (*Lolium* spp., *Sonchus oleraceus* and *Helminthotheca echioides*) and vineyard leaves (*Vitis vinifera*) on several locations in Western Portugal (Caldas da Rainha) and Southern Portugal (Beja, only in 2020).

The organs were observed under a stereomicroscope Leica 205C (Leica Germany) equipped with a NVI illumination, polarization filters and a MC190 HD camera. The leaves were also observed with an Olympus BX40 (Olympus, Japan) microscope in transmission mode (40 x) equipped with a darkfield set. Some photos were stacked to improve the depth of the field. The distinction between *A. alternata* and *A. arborescens* was done based on fungus morphology from undisturbed architecture on the host's surface. It was assumed that *A. tenuissima* and *A. alternata* are the same species (Woudenberg, 2015; Woudenberg et al., 2015).

3 RESULTS AND DISCUSSION

Spores and symptoms of BSD were identified in several perennial hosts such as *Pyrus communis* (Figure 1 A-O), *Ficus carica* (Figure 1 P-Q) and *Vitis vinifera*. Vegetables like *Capsicum annum* and *Solanum lycopersicum* (Figure 1 R) were also infected and produced spores similarly to several cover crops (*Lolium* spp., *Sonchus oleraceus* and *Helminthotheca echioides*). In all those species *S. vesicarium*, *A. alternata* and *A. arborescens* were identified. In case of *Lolium* spp. the *Alternaria* spp. present could be *A. alternata* or *A. infectoria*. The last one is quite common in cereal crops and humans, and it is hard to distinguish them based on morphology. As far as we know, the infections of

A. infectoria on plants were just reported for vegetables (Henriques, 2018; Kokaeva et al., 2018) and cereal species (Tralamazza et al., 2018).

In the initial leaf and fruit infection stage, it was possible to connect the symptoms to the spore responsible for the infection of the living tissues (Figure 1A-B). The cases where the relation was dubious were discarded. Thus, it was possible to establish a clear link between the symptoms associated with *S. vesicarium* and those caused by other *Alternaria* spp., namely *A. alternata* and *A. arborescens* on pear.

3.1 LEAF INFECTIONS

The infections caused by *S. vesicarium* and *Alternaria spp*. did not always develop a red halo on pear (Figure 1 D-G). Sporulation of *S. vesicarium* was never observed on pear leaves during the three years of the study. On the other hand, abundant sporulation on pear leaf lesions of *Alternaria* spp. was frequently found (Figure 1 C). The lesions on *V. vinifera* leaves produced abundant sporulation for both genera (Alternaria and Stemphylium, data not shown).

The presence of airborne *S. vesicarium* ascospores was often observed infecting both sides of the pear leaf. On the opposite, *S. vesicarium* conidia were rare to be observed on pear leaf blades. The reason behind this fact is unknown, as both spores can be collected from the atmosphere (Sobreiro et al., 2003).

In *S. vesicarium* and *Alternaria* spp. the spores produced multiple penetration hyphae that spread on host surface three to four times the spore length before the penetration site (Figure 1 A-B). In the leaves, direct penetration was the most frequent, but we also reported cases where leaf stomata penetration was clearly visible (data not shown).

The cover crops present in pear orchards were also infected by *S. vesicarium* and *Alternaria spp.* In the case of *Lolium* spp. the infections were observed on leaves and stems. Although green plant parts did not produce spores, as the plants dry out the infected areas started to produce *S. vesicarium* and *Alternaria spp*. conidia. Rain or dew stimulated the sporulation in these species (Sobreiro et al., 2003). *S. vesicarium* was also reported infecting several cover crops in pear orchards but not *Alternaria* spp. (Francisco, 2021).

Figure 1 – Infected pear leaf caused by *Stemphylium vesicarium* ascospore (A). Initial infection stage of *Alternaria* sp. in pear leaf (B). Sporulating *Alternaria alternata* in pear leaf lesion (C). *Alternaria* sp. pear leaf infection symptoms, red halo absent (D) or present (E). *S. vesicarium* pear leaf infection symptoms, red halo absent (F) or present (G). Pear dormant bud infected by *Alternaria arborescens* with mycelia and spores visible (H, I). *S. vesicarium* pseudothecia visible in a winter leaf disk collected from a pear orchard floor (J). Initial stage of a *S. vesicarium* formation with hyphae connected to a visible ascospore (K). Pear calyx infected by *A. alternata* with visible spores (L). Pear flower stigma and style infected by *A. arborescens* with visible spores (M). *A. alternata* sporulation visible on pear fruit collected at harvest (N). *S. vesicarium* on pear fruit observed at late harvest (O). *A. arborescens* abundant sporulation (P) and *S. vesicarium* high density sporulation on *Ficus carica* fruit in May (Q). Aspect of *S. vesicarium* spores growing on a *Solanum lycopersicum* fruit (R).

3.2 PEAR LITTER LEAVES

We confirmed that *S. vesicarium* overwinters as pseudothecia inside dead pear leaves at soil surface (Figure 1 J-K). This is the sexual stage of *S. vesicarium*, providing the fungus with the ability to adapt itself to the environment. One important source of *S. vesicarium* for Spring infection seems to be ascospores released by dead pear leaves. In this study we found many *S. vesicarium* ascospores landed over pear leaves and fruits. The ascospore dose over these organs was eight to 25 times higher than the number of *S. vesicarium* recorded conidia on the same organs. We hypothesise that *S. vesicarium* can also overwinter in other cover crops debris, but this claim needs confirmation.

The sexual stage of *A. alternata* and *A. arborescens* is unknown, but it is commonly visible inside cereal crops debris in the case of *A. infectoria* (Perelló and Sisterna, 2008).

3.3 FLOWER INFECTIONS

The flower infections linked to BSD occurred in the pear calyx, stigma, style, and filament but not in the anther. *S. vesicarium* just infected the flower calyx whereas *Alternaria* spp. infections were observed on the calyx, stigma, style (Figure 1 L-M) and filament. *S. vesicarium* lesions on flower calyx sporulated very rarely and accounted for less than 1% of the total flowers with visible infection symptoms. *Alternaria* spp. sporulation was frequent in the flower calyx, pistil, and filaments. In this study 26% of the pear flowers sampled exhibited infection symptoms. From the ones with symptoms, 0.6% were clearly *S. vesicarium*, 91.3% were *Alternaria* spp. and the remaining were unidentifiable.

The infection of cover crop flowers was observed in the plants of *Asteraceae* family, namely the Common sowthistle (*Sonchus oleraceus*) and the Bristly oxtongue (*Helminthotheca echioides*). The *Asteraceae* capitulum of these species was heavily infected by both *S. vesicarium* and *Alternaria* spp. and abundant conidial production was observed (data not shown).

3.4 FRUIT INFECTIONS

Several studies were conducted in Portugal on the pathogenicity and severity of *S. vesicarium* on pear (Dias, 2020; Francisco, 2021; Santos, 2019) and fungus identification on pome fruits (Silva, 2011).

Before June, the pear infections were more common in the area around the calyx. We observed that most spores were concentrated around this spot. After June, the infections were also common in the side of pear fruit facing the external and more illuminated sides of the trees. We observed the infections in apparently intact fruit epidermis and more often through the lenticels. The lesion initiated as less than 1 mm diameter brown spot, with or without red halo, and progressed slowly to reach as much as 25 mm diameter at harvest. This is in agreement with other studies performed in Portugal (Diogo et al., 2017; Sobreiro et al., 2003).

In the case of *S. vesicarium*, the lesions generally did not produce any spores, as previously mentioned for leaves. On the other hand, fruits with *Alternaria spp*. sporulating lesions were more likely to occur, especially on wounded pears.

At harvest time, the infected pears exhibited early maturation, turning yellow and falling to the ground about one week before harvest. The infection stimulated ethylene and CO_2 release in the infected fruits, which explains this behaviour (Almeida, 2015). As pear fruits turn yellow, *S. vesicarium* and *Alternaria* spp. infections produced mycelia with abundant sporulation. In some cases, the sporulation was observed only on the fallen pears. It was quite common the observation of *S. vesicarium* and *Alternaria* spp. on the same infected pear, individually or sharing the same lesion. The *S. vesicarium* sexual stage was absent in fallen fruits.

The observation of fig fruits exhibited strong sporulation for *S. vesicarium* and *Alternaria* spp. on lesions over 5 mm diameter (Figure 1 P-Q). The sporulation period was observed between late April and the end of July. The infected fig fruits remained on trees for weeks with visible sporulation before falling to the ground. Some infected fig fruits dried out and remained attached to the trees continuing to sporulate and resembling dark mummified fruits.

The infections of *S. vesicarium* and *Alternaria* spp. were also observed on bell pepper and tomato fruits. As soon as the fruit lesions reached 5 mm diameter, they sporulated very densely (data not shown).

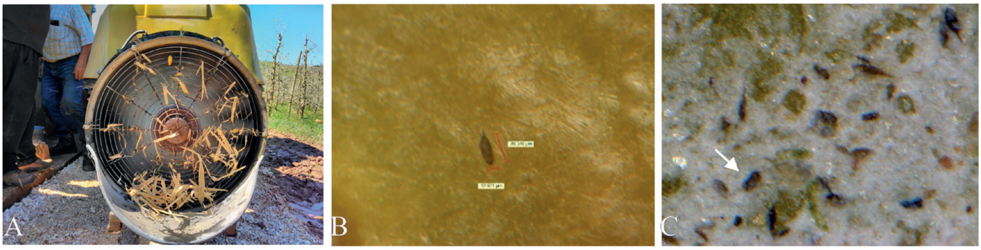
3.5 FLOWER BUD INFECTIONS

In wintertime (January and February) we observed many pear buds with internal tissues shrivelled and covered with dark areas. Inside many buds it was not possible to identify the cause of those symptoms. However, fungus mycelia were visible in 19% of the buds (average of the three years) (Figure H-I). The mycelia were also present within the bud scales. The *S. vesicarium* sporulation was never observed inside or outside the bud scales neither its pseudothecia. The *Alternaria* spp. was detected sporulating over the internal or external bud scales. Many flower buds with symptoms aborted or were not able to develop normal flowers in the Spring. Others presented far less flowers than normal, one to three per bud instead of the average seven. Regarding blossom blast of pear buds, the bacteria *Pseudomonas syringae* is the pathogen normally associated (Whitesides and Spotts, 1991). We observed that *A. alternata* and *A. arborescens* produced abundant sporulation in pear buds during winter. Each spring those spores were an important part of primary infections on pear tree, being responsible for important bud destruction. Those buds presented loose scales that fell easily. Some studies also suggested the *Alternaria* spp. as cause of blossom blast in pear (Wenneker et al., 2019).

3.6 AIR-BLAST SPRAYERS

The air-blast sprayers blow a strong air flow, sucking air from the rear and forcing the stream along with liquid pesticide, injected by the nozzles towards the pear canopy (Figure 2 A). In this study we measured the spore dose before (Figure 2 B) and after spray treatments (Figure 2 C). We observed 10 to 50 times more spores of *Alternaria* spp. and *S. vesicarium* deposited on pear fruit surface after the spray treatments. The *Alternaria* spp. spores were the most frequent (Figure 2 C). This means that air-blast sprayers were responsible for blowing up the spores when operating. The effect of this spore dose increment surely has a strong impact on future disease epidemic development.

Figure 2 – Air-blast sprayer commonly used to spray pear orchards in Portugal (A). *Alternaria* sp. conidia on pear fruit cuticula sampled before the spray treatment (B). Aspect of a spray droplet on a pear fruit surface with *Alternaria* spp. spores and a single *Stemphylium vesicarium* conidium, after a spray treatment (C).



3.7 BROWN SPOT DISEASE PATHOSYSTEM FOR PEAR ORCHARDS

One feasible way to represent the BSD pathosystem is shown in Figure 3. The BSD primary infections in Spring are caused by *S. vesicarium* ascospores released from the orchard floor, or conidia originated from fig trees in the orchard's neighbourhoods and dragged by the wind into the pear host. *S. vesicarium* ascospore release rate depends on light intercepted and water from dew or rain (Sobreiro et al., 2003). This event leads to Type A infections, where the leaves show an infection pattern with scattered spots, one or two per leaf. This pattern of infection is explained by the longest pathway of the spores in the atmosphere, before randomly landing on the susceptible tissues.

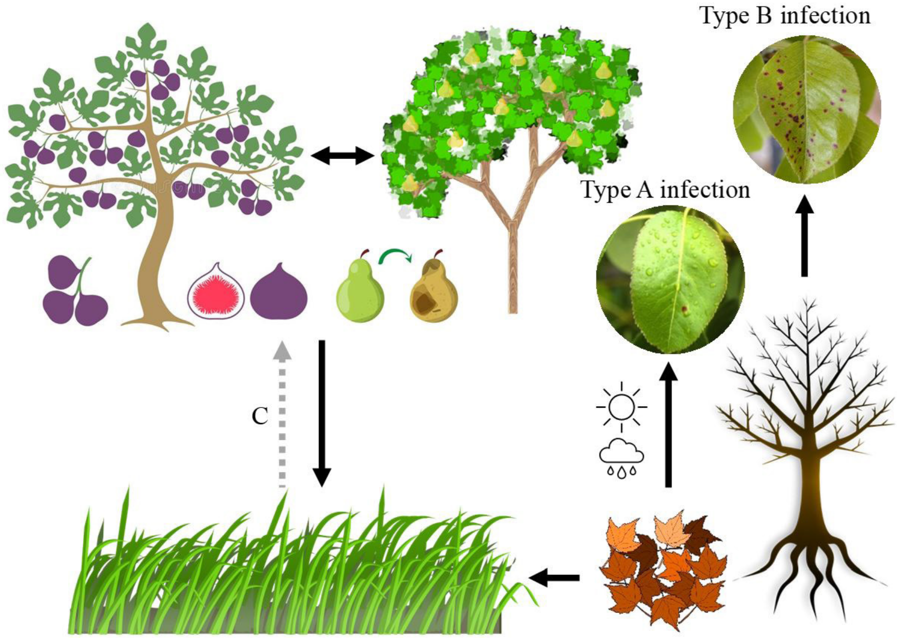
The Type B primary infection pattern happens when *Alternaria* spp. conidia originated on pear buds infect the nearby leaves. The infection source is remarkably close to the susceptible tissues, just centimetres apart, and the number of conidia released by lesion is extremely high. In this case, every pear leaf observed at the beginning of the season is heavily infected with five or more spots (Figure 3).

In Mediterranean climate, the orchard cover crops are fully established in October, as the rainfall begins to increase, and dry out in May or early June, in the absence of rain. In this scenario, deciduous species like pear, fig or vineyards are the most probable source of spores for cover crop *Alternaria* spp. infections. Dead leaves of pear trees discharge actively the ascospores at daylight into the atmosphere, providing the inoculum for *S. vesicarium* infection of perennial hosts or cover crops.

As *S. vesicarium* and *Alternaria* spp. spores are very heavy (falling about 3 m every 10 minutes in still air) (Woo et al., 2018) they to tend to settle down from the atmosphere very quickly and stay over the weeds or trapped between them. We observed that, in this case, man-made actions like spraying with air-blast sprayers could blow large amounts of those spores to the pear canopies (Figure 3).

It seems very unlikely that sporulating pear fruits fallen on the orchard's floor contribute to the disease epidemiology, due to the previous arguments (heavy spores, non-susceptibility of dry cover crops and the absence of spray treatments after harvest). This claim is supported by the small number of *S. vesicarium* conidia observed on the fruits before the spray treatments, and its increment afterwards. It has been reported that the removal of fruits and debris form pear orchard's floor presents a low impact in the number of *S. vesicarium* spores collected weekly on microscope glass slides, and thus in the disease epidemic progression (Constantino, 2019). Nevertheless, in other trial the mechanical removal of the orchard' fruits and debris after harvest influenced positively the spore deposition over the pear canopies (Afonso, 2021).

Figure 3 – Pathosystem representation of the Brown Spot Disease. Primary infection sources are the ascospores of *Stemphylium vesicarium* from the dead leaves on the pear orchards floor (infection type A) or conidia from *Alternaria* spp. overwintering mycelia in pear buds (infection type B). Other source of inoculum could be the fig trees in the orchard's vicinity for *Alternaria* spp. or *S. vesicarium*. Cover crops are most likely to be infected by spores from perennial or from dead debris on orchard floor. The anthropomorphic actions like use of air-blast sprayers could lead to the high spore dose on fruits surface from multiple inoculum sources (C).



4 CONCLUSION

In summary the main insights into Brown Spot Disease in agroecosystems are:

- *Stemphylium vesicarium* and *Alternaria* spp. exhibit two types of similar symptoms on pear leaves or fruits: brown spots with or without red halo. The symptoms cannot be interpreted as the signs of those pathogens. Therefore, the sample judging by naked eye can be misleading.
- *S. vesicarium* overwinters as pseudothecia on dead pear leaves on the orchard floor that actively discharge ascospores during daylight when wetted by rain or dew. *S. vesicarium* pseudothecia seem not to develop in pear buds.
- *A. alternata* and *A. arborescens* sexual stage is unknown and does not produce ascospores. Mycelium of *Alternaria* spp. sporulate in pear flower buds, causing blossom blast and contributes significantly to primary infections of BSD.
- *Alternaria* spp. conidia are more frequent on pear leaf blades or fruit surface than *S. vesicarium* ascospores and conidia. In the same tissues, *S. vesicarium* ascospores are more frequent than conidia.

- Pear flowers are infected with *S. vesicarium* and *Alternaria* spp. *S. vesicarium* conidia are rarely produced in pear flowers whereas abundant conidia of *Alternaria spp.* are produced in all pear flower parts, excluding the anthers.
- Fruit lenticels are important openings for *S. vesicarium* and *Alternaria* spp. infection.
- The green tissues of cover crops infected by BSD do not sporulate. The *Asteraceae* capitulum produces abundant *S. vesicarium* and *Alternaria* spp. conidia, late in Spring, when the cover crops dry out.
- The use of herbicides or mowing grass below 10 cm height is beneficial to *S. vesicarium* and *Alternaria spp*. sporulation, since dead cover crops tissues, when exposed to sunlight, provide large amounts for fungi growth.
- The air-blast sprayers increase the number of spores of *S. vesicarium* and *Alternaria* spp. that land over the pear canopies, 10 to 50 times per spraying.
- Brown Spot Disease is highly influenced by anthropomorphic actions and climate changes in ecosystems. A global approach is needed to reestablish the ecosystem equilibrium to manage this disease.

5 FUNDING

This work received no external funding.

6 CONFLICTS OF INTEREST

The author declares no conflict of interest.

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SOBRE O ORGANIZADOR

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