Brown spot of pear

Stemphylium vesicarium

Diana Pimentel

InnovPlantProtect

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1 Abstract

Brown spot of pear (BSP) disease is caused by the fungus *Stemphylium vesicarium* and it has high economic relevance in pear-producing areas in Europe. Besides pear, this pathogen also causes diseases in other plants, such as garlic, onion, asparagus, alfalfa, mango and soybean. *S. vesicarium* was first reported in pear in 1975 in Italy and is currently spread across all continents, however, it was only reported in pear in Europe. In Portugal, the incidence of brown spot specially in ‘Rocha’ pear has been increasing and was responsible for the loss of 25% of ‘Rocha’ pear production in 2015. Symptoms are characterized by necrotic lesions on fruit, leaves, petioles, and twigs. In consequence, infected fruits lose their commercial value and severe attacks can cause premature defoliation and fruit abscission prior to harvest. BSP symptoms are usually observed in late spring and progressively increase until harvest. For instance, temperature and humidity play an important role in disease development and different forecasting models have been developed based on these environmental parameters, including BSPcast and PAMcast models. BSP disease management is based on preventive fungicides applied during the pear growing period, either at a fixed schedule or according to a forecasting system. Nevertheless, fungicides efficacy is low under high disease pressure, due to inoculum presence, favourable environmental conditions, or to the high susceptibility of pear cultivars.

2 Resumo

A estenfiliose da pereira é causada pelo fungo *Stemphylium vesicarium* e tem grande importância económica nas áreas de produção de pêra na Europa, podendo atingir uma perda de até 90% da produção. Para além da pêra, este fungo também afecta outras plantas, como alho, cebola, espargos, alfalfa, manga e soja. No entanto, observa-se que as estirpes patogénicas destes hospedeiros não são patogénicas na pereira. Para além disso, o fungo pode também sobreviver como saprófita em resíduos vegetais nos pomares. O *S. vesicarium* foi detectado pela primeira vez em 1975 na região de Emilia-Romagna em Itália. A partir daí foi surgiram novos surtos em Espanha (1984), França (1987), Portugal (1996), e Holanda (1997). Actualmente está presente em vários países espalhados em todos os continentes. Em Portugal tem-se registado um aumento da estenfiliose, especialmente na região do Oeste onde já tem carácter epidémico. Em 2015, foram registados prejuízos de 25% da produção de pêra ‘Rocha’.

Os sintomas caracterizam-se por manchas necróticas nos frutos, folhas, pecíolos e pedúnculos. Nas folhas os primeiros sintomas aparecem de Abril a princípios de Junho. Os frutos perdem valor comercial e, no caso de infecções mais severas, pode ocorrer a queda prematura de folhas e frutos antes da maturação. O fungo sobrevive durante o Inverno na forma de pseudotecas nos restos vegetais presentes no solo do pomar. Em condições ambientais favoráveis, os ascósporos (esporos sexuados) são libertados e dispersos pelo vento e pela chuva, desde fevereiro a junho, causando as infecções primárias. Os conídios (esporos assexuados) são produzidos de Abril até Setembro sendo as condições óptimas temperaturas entre 20ºC e 25ºC e humidade relativa elevada. As temperaturas óptimas para infecção situam-se entre 20ºC e 25ºC com um período mínimo de humectação de 6 horas.

O controlo da estenfiliose passa pela aplicação de tratamentos preventivos com fungicidas e por medidas culturais. As medidas culturais incluem a remoção de frutos e árvores com sintomas, remoção do material vegetal infectado presente no chão, promover a drenagem dos solos e o

arejamento das copas das árvores, aplicar uma fertilização adequada evitando tanto carências nutritivas como excesso de azoto, manter o coberto vegetal na entrelinha baixo e manter a linha das árvores limpa de infestantes. Vários modelos de previsão foram desenvolvidos, nomeadamente os modelos BSPcast e PAMcast, que têm sido utilizados para determinar o melhor momento para aplicação dos fungicidas. No entanto, a eficácia dos fungicidas não é muito elevada quando existe uma elevada pressão para o desenvolvimento da doença, como elevada presença do inóculo, condições ambientais favoráveis, ou variáveis muitos susceptíveis. Para além disso, vários estudos referem o desenvolvimento de resistência a vários fungicidas.

3 Introduction

Stemphylium vesicarium (Wall.) E. Simmons is the causal agent of the brown spot of pear (BSP) which has high economic relevance in certain pear-producing areas in Europe (Llorente and Montesinos, 2006). *S. vesicarium* was first reported in 1975 in Italy (Emilia-Romagna region), thereafter it was observed in Spain (Girona) in 1984 and France (Rhône) in 1987. In addition, new outbreaks were detected in several European countries, including Portugal (1996) and Netherlands (1997), and is now widely spread across all the continents. In Portugal the incidence of brown spot specially in 'Rocha' pear has been increasing and, between 2014 and 2016, it led to losses of about 30 million euros, according to INIAV in 2017 at the Fruit Growing Technical Conferences. In 2015, BSP was responsible for the lost of 25% of 'Rocha' pear production (Diogo et al., 2017). Besides, *S. vesicarium* also causes diseases in other fruit trees, such as mango (Johnson et al., 1990), and in herbaceous crops, such as garlic, onion, asparagus, alfalfa, and soybean (Aveling and Snyman, 1993; Darrag et al., 1982; Falloon, 1987; Lamprecht et al., 1984; Prados-Ligero et al., 1998). BSP symptoms include necrotic lesions on pear fruits, leaves and shoots. Symptoms on young fruits are usually located on the calyx, whereas on mature fruits, necrotic spots are observed in the equatorial zone (Llorente et al., 2012). Fruits lesions gradually expand and a secondary infection by saprophytic fungi, such as *Alternaria* sp., may produce fruit rotting (Llorente et al., 2012). Infected fruits lose their commercial value and severe attacks can cause premature defoliation and fruit abscission prior to harvest. BSP symptoms are usually observed in late spring and progressively increase until harvest.

S. vesicarium has both sexual and asexual reproduction phases in its biological cycle, leading to the production of two kinds of inoculum: the ascospores as sexual inoculum, which are produced by the ascomycete *Pleospora allii*, and the conidia as asexual inoculum, that are produced by the deuteromycete *S. vesicarium*. Inoculum monitoring in pear orchards is mainly achieved through spore traps and species identification is based on conidial morphology. However, several species of *Stemphylium* coexist in pear orchards, but only isolates identified as *S. vesicarium* were shown to be pathogenic on pear and consequently, direct measurements of the airborne inoculum using spore traps may overestimate the actual pathogen population (Puig et al., 2015).

Differences in susceptibility to BSP disease have been observed among pear cultivar. Cultivars Passe Crassane, Abate Fétel, Alexandrine, and Conference are very susceptible; Kaiser, Rocha, and Winter Nellis are moderately susceptible; and Williams, Blanquilla, Beurre Hardy, Louis Bonne, Grand Champion, and Highland are low or non-susceptible (Llorente and Montesinos, 2006). Differences in susceptibility can be explain by the production of two host-specific toxins, SV-toxin I and II (Singh et al., 2000, 1999). Studies reveal that most of the host-specific toxins produced by

S.versicarium have low or no measurable toxicity to mammals (Stricker et al., 2021). Moreover, differences in pathogenicity has been reported for several *S. vesicarium* isolates (Köhl et al., 2009a). Additionally, *Stemphylium* isolates originating from onion and asparagus crops were shown to be not-pathogenic to pear and only isolates originating from pear orchards, including isolates from dead grass leaves, were pathogenic on pear leaves or fruits (Köhl et al., 2009a).

The first draft genome of *S. versicarium* (strain 173-1a-13FI1M3) isolated from pear was sequenced in 2019 with 38.66 Mb in length (Gazzetti et al., 2019). Two more assemblies are available from strains (On16-63 and On16-391) isolated from onion (Sharma et al., 2020).

4 The pest and its biology

4.1 Taxonomy

Name: *Stemphylium vesicarium* (Wallr.) E. Simmons

Synonyms: *Pleospora allii* (Rabenh.) Ces. & De Not. (teleomorph)

Taxonomic tree:
Domain: Eukaryota
- Kingdom: Fungi
  - Phylum: Ascomycota
    - Subphylum: Pezizomycotina
      - Class: Dothideomycete
        - Order: Pleosporales
          - Family: Pleosporaceae
            - Genus: *Stemphylium*
              - Species: *Stemphylium vesicarium*

Common names: brown spot of pear (english), estenfiliose da pereira (portuguese), mancha negra del peral (spanish).

EPPO code: PLEOAL (Pleospora allii).

Based on molecular and chemotaxonomic analysis, several authors consider that the species *S. alfalfae*, *S. herbarum*, *S. sedicola*, *S. tomatonis* and *S. vesicarium* are synonyms (Câmara et al., 2002; Olsen et al., 2018; Woudenberg et al., 2017).

S. vesicarium conidia are dark, oblong to oval, multicellular, and with one to five transverse septa and one to two series of longitudinal septa, constricted at one, or more commonly three, of the major transverse septa (Llorente and Montesinos, 2006). In general, conidia range size is from 21 to 48 µm in length and 10 to 22 µm in width, being the length-to-width ratio of 1.5 to 2.7 for conidia developed in host tissue and 2.5 to 3.0 in growth media (Llorente and Montesinos, 2006). Conidia are formed in the aerial mycelium, in conidiophores which are erect, brown, and with only one terminal conidium. Aerial mycelium is filamentous, sparse, and hyaline (Llorente and Montesinos, 2006). In adverse conditions, the fungus develops sexual structures named pseudothecia with asci inside. Pseudothecia are brown to black, coriaceous, globose and ostiolate, with an average diameter of 100 to 500 µm (Llorente and Montesinos, 2006). Asci are bitunicate, cylindric-clavate (131 × 26 µm), with eight ascospores inside. Ascospores are yellow-brown, ellipsoidal or oblong to clavate (32 × 14 µm), containing three to seven transverse septa and one longitudinal septum in each of most initial transverse divisions of the spore, plus secondary ones in the transverse sub-divisions (Llorente and Montesinos, 2006).

4.2 EU and PT regulatory status

S. vesicarium is not regulated in European Union. However, following the severe damages that have been occurring in the 'Rocha' pear, specially in 2015, it was established by order no. 11400/2016, published on Republic Diary No. 184, Series II of 2016-09-23, a working group whose mission is to design an action plan to control the BSP disease. This group is led by INIAV, with the participation of COTHN, ANP, DGAV and DRAPLV. Every week is released the monitoring results of BSP disease on the Oeste region, which can be found here, in which is included the average number of conidia, of ascospores and both per week per orchard, and the comparison of the average number of spores against the average of the four years of monitoring.

4.3 Disease distribution

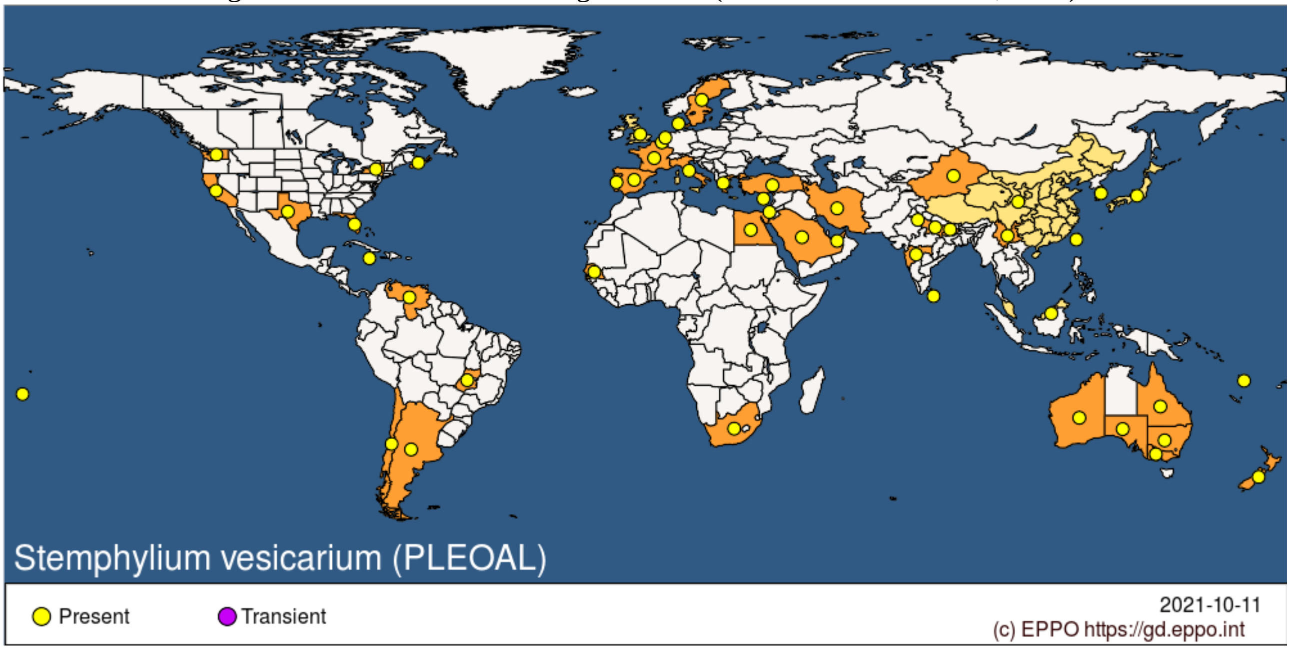
Brown spot of pear was first reported in 1975 in Italy (Emilia-Romagna region) in the Abate Fetel variety (Ponti et al., 1982). In 1984, was detected in Spain (Girona), thereafter in 1987 in France (Bouches du Rhôde). Subsequently, it has been reported in several Portugal (1996), Netherlands (1997) and Belgium (2002) (Llorente and Montesinos, 2006). Nowadays *S. vesicarium* is widely spread across all continents (CABI, 2006):

- Europe: Belgium, Cyprus, France, Greece, Italy, Netherlands, Portugal, Spain, Sweden, UK;
- Asia: China, India (Bihar, Haryana, Maharashtra), Iraq, Israel, Japan, Korea Republic, Malaysia, Saudi Arabia, Sri Lanka, Taiwan, United Arab Emirates;
- Africa: Egypt, Senegal, South Africa;
- North America: Canada (Nova Scotia), USA (California, Florida, New York, Texas, Washington);
- Central America and Caribbean: Jamaica;
- South America: Argentina, Brazil (Goias), Chile, Venezuela;
- Oceania: Australia (New South Wales, Queensland, South Australia, Victoria), New Zealand, Tonga, Vanuatu.

Despite the wide distribution, *S. vesicarium* was only reported in pear in Europe (Diogo et al., 2017; Sousa et al., 2004).

In Portugal, BSP was first confirmed in 1996 in Rocha and Passe Crassane varieties, nevertheless similar symptoms were observed previously (Diogo et al., 2017). This disease was initially detected in Alcobaça and Bombarral, and then it spread to all Oeste region (Torres Vedras, Bombarral, Cadaval, Lourinhã, Caldas da Rainha, Alcobaça, Nazaré, Porto de Mós, Batalha and Leiria) (Sousa et al., 2004).

Figure 1: Distribution of fire blight disease (EPPO Global Database, 2021).



4.4 Disease cycle

The disease cycle is characterized by two kinds of inoculum: the sexual inoculum (ascospores of *Pleospora allii*) and the asexual inoculum (conidia of *S. vesicarium*). The sexual phase occurs mainly during autumn and winter when the fungus overwinters as pseudothecia of *P. allii* in dead plant material, including not only dead pear leaves and fruits but also tissues of herbaceous plants (Llorente and Montesinos, 2006; Rossi et al., 2005). On the other hand, the asexual phase occurs during the vegetative period of pear growth during spring and summer (Llorente and Montesinos, 2006). The *S. vesicarium* life cycle is characterized by two different stages (Figure 1): a pathogenic phase on the aerial pear tree organs during the period of pear growth and a saprophytic phase corresponding to permanent colonization of the plant debris over the year (Llorente et al., 2012).

The maturation of pseudothecia begins with paraphyses filling the lumen of the pseudothecium, followed by asci formation and differentiation of eight ascospores in each ascus. Pseudothecia develop under high relative humidity (RH >98%) at optimum temperatures between 10 and 15°C (Llorente and Montesinos, 2004). Below 5°C, the rate of maturation of pseudothecia decreases and do not mature at or above 25°C (Llorente and Montesinos, 2004). The first mature pseudothecia are mainly observed from the middle of December to the end of February, and most pseudothecia have asci fully developed from the middle of January to the end of April (Llorente and Montesinos, 2006). Then, from February to early June, ascospores are produced and released by rain or heavy dew on the pear leaf litter (Llorente and Montesinos, 2006). A second ascospore production period also occurs between August and October (Llorente et al., 2012). Since BSP symptoms normally appear during June when the level of airborne ascospores is low, their role as inoculum is probably the saprophytic colonization of pear debris on the orchard floor, beginning the asexual phase under warmer environmental conditions. The airborne conidia produced by the resulting mycelium then infect pear trees during the growing period (Llorente et al., 2012). Conidia are produced from April to November with a maximal release from July to September. Several studies support the hypothesis of the inoculum being produced on plant debris at the orchard ground or in neighbouring orchards, since the removal of soil inoculum reduces the disease level and the dispersal patterns are shorter (Llorente et al., 2012; Rossi et al., 2008). Moreover, DNA of pear-pathogenic *S. vesicarium* was detected at high concentration in both fallen pear residues and weeds and grass leaves (Köhl et al., 2013). In portuguese orchards, isolates from *Rumex crispus* L., *Picris echioides* L., *Oxalis pes-caprae* L., *Epilobium* sp. And *Sonchus* sp., which are common weeds in 'Rocha' orchards, were observed to cause lesions in 'Rocha' fruits 15 days after inoculations (Isidoro and Azevedo, 2005).

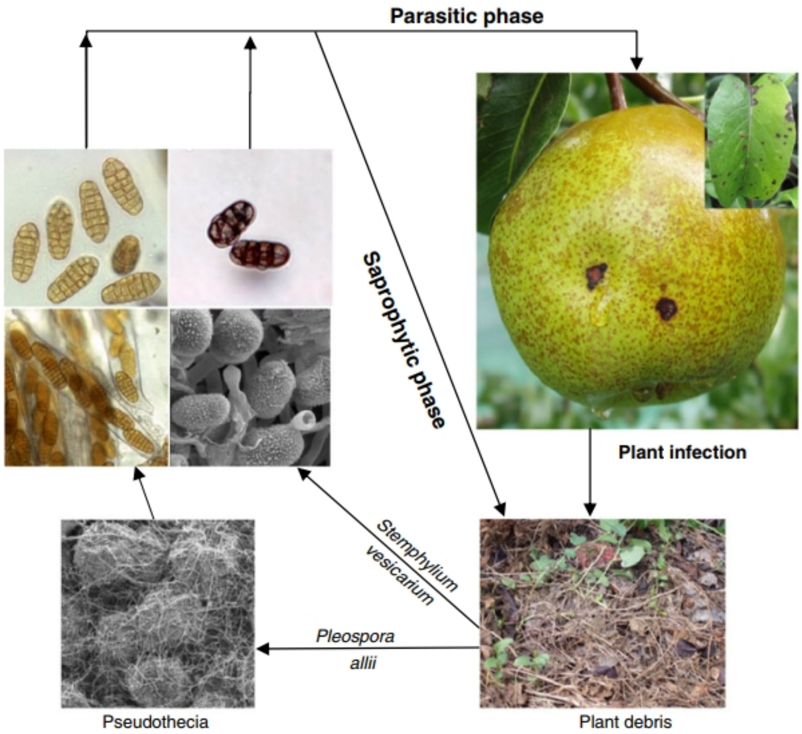


Figure 2: Life cycle of *Stemphylium vesicarium* and *Pleospora allii* on pear orchards (Llorente et al., 2012)

5 Host Plant Target population

5.1 Host range and main hosts

S. vesicarium cause diseases on a wide range of different hosts, namely pears (Ponti et al., 1982), mango (Johnson et al., 1990), garlic (Prados-Ligero et al., 1998), onion (Aveling and Snyman, 1993), asparagus (Falloon, 1987), tomato (Porta-Puglia, 1981), alfalfa (Lamprecht et al., 1984), soybean (Darrag et al., 1982), parsley (Koike et al., 2013), sunflower (Arzanlou et al., 2012) and aster (Ichikawa and Sato, 1994). *S. vesicarium* can also live as a saprobe on plant debris.

Rumex crispus L., *Picris echioides* L., *Oxalis pes-caprae* L., *Epilobium* sp. And *Sonchus* sp., which are common weeds in 'Rocha' orchards, were shown to be secondary hosts of pear-pathogenic *S. vesicarium* (Isidoro and Azevedo, 2005).

Host list: *Allium cepa, A. sativum, Asparagus officinalis, Aster spp., Glycine max, Helianthus annuus, Mangifera indica, Medicago spp., Petroselinum crispum, Pyrus communis, Solanum lycopersicum.*

5.2 Environmental suitability

Both temperature and humidity are essential for disease development. Free water is needed to initiate conidial germination (Montesinos and Vilardell, 1992). Moreover, the optimal temperature for germination ranges from 15 to 32°C with a very high rate of germination and mycelial growth, in one hour 50% of the conidia germinate under optimal conditions (Montesinos and Vilardell, 1992). The optimal temperature for disease establishment in susceptible cultivars are between 20 and 25°C, with a maximum at 22.6°C on fruits of cv. Passe Crassane and 21.1°C on leaves of cv. Conference (Llorente and Montesinos, 2006). Under these conditions, a 6-h wetness period is enough to start infections, and infection probability increase with increased wetness duration (Montesinos et al., 1995b). At low temperatures (16°C), the symptoms can appear after 4 days after infection, however, at high temperatures (25 to 30ºC) and with 16-h wetness period, the symptoms can appear only after 48h (Sousa et al., 2004).

High relative humidity (RH ≥98%) is vital to the development of pseudothecia during the winter, with an optimal temperature from 10 to 15°C (Llorente and Montesinos, 2006). Low RH (60%) during periods of interrupted wetness stops the infection process (Llorente and Montesinos, 2002). On the other hand, high RH (96%) during the intervening dry period slowed infection rate when the duration of the interruption was less than 6 h, but the infection rate increased with the length of the wetness period interruption (Llorente and Montesinos, 2002).

5.3 Spread capacity

The ascospores and, in less frequency, the conidia produced in the winter and in the beginning of the spring on the leaf's debris on the orchard ground, under favourable conditions of temperature and humidity are blown away by wind and rain and settle down on young leaves and fruits, beginning the infection (Sousa et al., 2004). If the conditions are still favourable, primary infections give origin to conidia in the necrotic tissues. These conidia are spread by the wind infecting new leaves and fruits and germinate under humid conditions (Sousa et al., 2004).

5.4 Risk factor identification

S. vesicarium infection is an important fungal disease of pear. Identification and estimation of risk factors are essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infection by a disease in the area of interest.

Main risk factors are the presence of high levels of inoculum, temperature and wetness periods, and susceptibility of plant host. The incidence of BSP disease is higher in pear orchards with a lawn of herbaceous plants than in bare soils (Rossi and Pattori, 2009), because it increases the levels of inoculum. Soil orchard management strategies (mechanical cultivation or natural cover) can influence the incidence and prevalence of *S. vesicarium* infection, being mechanical cultivation more useful in inoculum reduction than natural grass cover (Reis et al., 2021). It was also observed that the BSP incidence history has high impact in disease prevalence (Reis et al., 2021).

Regarding environmental conditions, the optimal temperature for disease establishment in susceptible cultivars are between 20 and 25°C, and under these temperatures, a 6-h wetness period is sufficient to start infections (Montesinos et al., 1995b).

Susceptibility of plant host also plays an essential role in BSP disease incidence. Among cultivars there are differences in susceptibility at the same environmental conditions: Passe Crassane, Abate Fétel, Alexandrine, and Conference are the most susceptible; Kaiser, Rocha, and Winter Nellis are moderately susceptible; and Williams, Blanquilla, Beurre Hardy, Louis Bonne, Grand Champion, and Highland are slightly or non-susceptible (Llorente and Montesinos, 2006). Susceptibility to BSP also depends on phenological stage and pear cultivar. The susceptibility of fruit and leaves decreases logarithmically with physiological age (Montesinos et al., 1995a). Young and chlorotic leaves and young fruits are more susceptible to infection (Sousa et al., 2004). Nevertheless, at the ripening stage, when firmness and acids and starch contents decrease and the sugar content increase, the fruits became more susceptible again (Sousa et al., 2004).

5.4.1 Predictive models

Different forecasting models have been developed to determine the effect of environmental parameters on different stages of the biological cycle of the pathogen or the disease. The most used are the BSPcast and the PAMcast models.

BSPcast model

The BSPcast model (Brown Spot of Pear forecasting system) was developed to predict infection risk on pear, both in leaves and fruits, based on an empirical model (Montesinos et al., 1995b). This model determines the effect of daily wetness duration (*W*) and mean air temperature during wetness periods (*T*). Optimal conditions for infections are more than 24 h of continuous wetness at 22.5°C. Polynomial equations were developed for fruit and leaf infection using $\log_{10}$ transformation of severity of the BSP disease as lesions/leaf (*S*):

$$\log_{10} S = -1.70962 + 0.0289\,T + 0.04943\,W + 0.00868\,TW - 0.002362\,W^2 - 0.000238\,T^2 W$$

Because the maximum daily disease severity (*S*) predicted by the model was 3.79, a relative daily infection risk (*R*) is calculated as:

$$R = \frac{S}{3.79}$$

Every day a relative daily infection risk is calculated and then a cumulative daily infection risk (CR) is calculated by summing *R* values for the past 3 days. The CR is used to predict the time for spraying fungicides. Moreover, the effect of interrupted wetness periods and relative humidity during the interruption was incorporated into the BSPcast model. This effect should be considered interrupted if the length of interruption is ≥3 h at low relative humidity (Llorente and Montesinos, 2002). BSPcast model has been evaluated and validated during several years in Spain and Italy (Llorente et al., 2000; Montesinos et al., 1995b), and it has also been tested with success in Belgium, The Netherlands, and Portugal (Llorente and Montesinos, 2006). BSPcast model is currently used to schedule fungicide sprays. The average savings in number of fungicide sprays applied using BSPcast are around 20 to 70% when compared with the fixed-spray schedule (Llorente and Montesinos, 2006).

PAMcast model

The PAMcast model (*Pleospora Allii* Maturation forecast) was developed to relate environmental conditions to the development of pseudothecia of the sexual phase, based on temperature and relative humidity during the winter (Llorente and Montesinos, 2004). Assuming that P. alli development is dependent on temperature (assuming 0°C as the base temperature), this model quantifies the effect of cumulative degree days (CDD) in maturation process of pseudothecia, estimating the percentage of mature pseudothecia.

$$\ln\left(\frac{1}{1-y}\right)=0.12550+0.005048\,x$$

where *y* is the proportion of mature pseudothecia and *x* is CDD.

The first mature pseudothecia usually are observed between December and February in Europe, depending on orchard conditions. Most pseudothecia are fully mature after 750 CDD and the release of mature ascospores is related to the rain or heavy dew. The PAMcast model has been evaluated under different orchard conditions in Spain. The model can be used for determining when pseudothecia and ascospores are mature, and consequently to predict when ascospores are released in the field. In addition, it can be used to establish the date to begin running the BSPcast system for scheduling fungicide sprays.

6 Detection and identification

6.1 Detection and identification in the field

Disease symptoms can appear in all vegetative stage in leaves, fruits, twigs and petioles (Sousa et al., 2004). The first symptoms on fruit are observed at fruit set (from April to June) and begin as circular and brown spots with a range from 1 to 2 mm in diameter, and sometimes surrounded by a red halo (Diogo et al., 2017; Llorente and Montesinos, 2006). On mature fruit, spots increase to 10 to 20 mm and, if spots are invaded by saprophytic fungi such as *Alternaria* spp., internal rot may occur (Llorente and Montesinos, 2006). The symptoms appear mainly on the region more exposed to direct sun light (Sousa et al., 2004). Leaf symptoms are first observed from late April to May, and more frequently in June, and consist of brown spots, ranging from 1 to 3 mm in diameter (Llorente and Montesinos, 2006). With disease progression, the central part of the spot became coalescent, greyish and brownish (Sousa et al., 2004). Brown spots develop along the main and secondary veins, on the margins and at the end of the blade, with a triangular or trapezoidal shape (Sousa et al., 2004). Severe outbreaks can result in premature defoliation, and fruit abscission prior to harvest (Llorente and Montesinos, 2006). BSP disease can also affect both petioles and peduncles, appearing as small and brown spots, without modification of the tissues' structure (Sousa et al., 2004).

6.2 Detection and identification in the lab

The detection and identification of *S. vesicarium* conidia and *P. allii* ascospores, as well as the assessment of inoculum levels, are normally accomplished through spore trap devices and optical microscope observations. Species differentiation can be performed based on the morphological traits (Simmons, 1969), nevertheless, *S. vesicarium* shares morphological similarities with other *Stemphylium* species, such as *S. astragali*, *S. herbarum*, *S. alfalfae*, *S. majunsculum*, *S. gracilariae*, *S. beticola*, *S. eturmiunum*, *S. simmonsii* and *S. botryosum* (Câmara et al., 2002). Molecular markers can also be used for differentiation of pathogenic and non-pathogenic *S. vesicarium* isolates in natural populations (Köhl et al., 2009b, 2009a; Llorente et al., 2010; Puig et al., 2015). Moreover, chemotaxonomic analysis via HPLC-UV-M of *Stemphylium* genus reveal some species-specific metabolites, which can be used for identification (Olsen et al., 2018).

7 Economic and social impact

In Portugal, "Rocha" pear (*Pyrus communis* L. cv Rocha), whose production is mainly concentrated in the west region of the country, has huge economic impact accounting for 95% of national pear production. According with the National Association of Rocha pear Producers (ANP), the average production of Rocha pear is around 173 000 tons. The incidence of BSP disease has been increasing causing significant yield reduction. In 2015, BSP disease caused a loss of 25% of "Rocha" pear production (Diogo et al., 2017). It was also estimated that between 2015 and 2017 the production losses were around 29 704 million euros (Diogo et al., 2017). According to APAS (*Associação dos Produtores Agrícolas da Sobrena*), the average disease incidence in Portugal is 15%, with annual losses of 17 325 000€ to the producers and 49 500 tonnes in production. It was also observed that in drier years the disease incidence is higher than rainy years (APAS, personal communication, November 2021).

Table 1: Economical impact of Brown Spot of Pear in Portugal

	APAS (2021) [1]
Average incidence	15%
Annual economical losses for the producers (€)	17325000
Annual economical losses for the value chain (€)	66825000
Annual production losses (tonnes)	49500
Annual cost of control measures (pruning, phytochemicals, etc.; €)	22000000

8 Available control/prevention methods

BSP disease management is based on preventive fungicides applied several times during the pear growing period, either at a fixed schedule or according to a forecasting system. Nevertheless, fungicides efficacy is low under high disease pressure, due to inoculum presence, favourable environmental conditions, or to the high susceptibility of pear cultivars (Llorente et al., 2012).

8.1 Chemical

Chemical control is the most efficient method to control BSP disease and is mostly preventative. The most commonly used fungicides are dithiocarbamates (thiram, mancozeb), dicarboximides (procymidone), strobilurins (kresoxim-methyl, trifloxystrobin, pyraclostrobin), captan, chlorothalonil, tolifluanide, tebuconazole, fludioxinil, and difenoconazole (Llorente and Montesinos, 2006). In orchards moderately to highly affected by the disease, 15–25 fungicide sprays are required to keep low levels of disease incidence in fruits (Llorente et al., 2012). Resistance to dicarboximides, fludioxonil and strobilurins have been reported (Alberoni et al., 2010, 2008, 2005; Gazzetti et al., 2015), and an integrated approach might be more efficient, including

1 Calculated by João Azevedo (from Associação dos Produtores Agrícolas da Sobrena) based on the assumptions:
- Total orchard area in Portugal – 11 000 ha;
- Average productivity – 30 ton/ha
- Average value paid to producer – 0,35€/kg
- Average value paid by retailers – 0,85€/kg
- Retail selling margin – 100%
- Average value for the remaining value chain – 1,35€/kg

preventive measures to reduce the presence of inoculum, complemented with preventive chemical and/or biological control (Diogo et al., 2017; Llorente et al., 2012). Recently, propolis extracts have been suggested as potential control strategy to BSP disease, since *in vivo* assays with propolis extracts in artificially inoculated "Rocha" pears showed that disease incidence decreased up to 25% and the lesions diameter up to 57% (Loebler et al., 2020).

In Portugal, the fungicides approved by DGAV against BSP in pear are boscalid + pyraclostrobin (Bellis), cyprodinil + fludioxinil (Serenva, Switch 62,5 WG), cyprodinil + tebuconazole (Benelus), cooper (Calda Bordalesa Selectis), kresoxim-methyl (Stroby WG, Valkrom, Ksar, Decibel, Quimera, Sugoby), kresoxim-methyl + difenoconazole (Ksar Max, Colombo), dithianon + pyrimethanil (Faban 500 SC), fluazinam (Banjo), fluopyram + tebuconazole (Luna Experience), fluxapyroxad (SERCADIS 30 SC), tebuconazole (Libero Top, Fezan, Fox Plus, Tebutop WG, Mystic 25 WG), trifloxystrobin (Flint, Consist, Safira), ziram (Zidora AG, Zico, Thionic WG) (https://sifito.dgav.pt/divulgacao/usos).

8.2 Biological

Several biological control agents have been studied against BSP disease, nevertheless the efficacy in field conditions was very low. *Trichoderma koningii* and *T. viride* has been evaluated on trees, however no disease reduction was observed (Ponti et al., 1993). In another study, 400 strains of *Pseudomonas fluorescens* and *Pantoea agglomerans* were screened as possible biocontrol agents against *S. vesicarium*, nevertheless, low efficacy was observed on field trials (Montesinos et al., 1996; Montesinos and Bonaterra, 1996). Application of *Trichoderma* sp. formulations on leaf debris in the orchard floor was effective in decreasing the overwintering inoculum of *P. allii*, and the efficacy increase when applied at the beginning of pseudothecia maturation (Llorente et al., 2006). Moreover, application of *Trichoderma sp.* products also shown promising results restricting the conidia production by *S. vesicarium* throughout the growing season (Rossi and Pattori, 2009).

In a recent study, six biological control agents (BCAs) approved in Europe based on *Bacillus subtilis* and *Trichoderma* spp. were tested and it was shown that *Bacillus subtilis* Bs1 (Serenade Max from Bayer Crop Science) and *Trichoderma* spp. Tr1 (Tusal from Certis Europe) and Tr2 (Trianum-P from Koppert Biological Systems) could reduce fungal inoculum during the pear vegetative period by at least 45–50%, and *Trichoderma* spp. Tr1 and Tr2 were able to reduce the fungal overwintering inoculum by 80% to 90% (Moragrega et al., 2021).

On other hosts, *Trichoderma asperellum* was shown to be a potential biological control agent of *S. vesicarium* on onion, reducing the severity of leaf blight disease and the foliar damage (Zapata-Sarmiento et al., 2020).

In Portugal, none biological control agent is approved against BSP disease in pear or another host. Nevertheless, *Bacillus subtilis* and *Trichoderma* spp. formulations are approved to other diseases.

8.3 Cultural

Shred and remove leaves from the orchard ground during autumn are effective in reducing the ascospore inoculum (Llorente et al., 2006; Llorente and Montesinos, 2006). Trees and fruits with visible symptoms should be removed from the orchard (Diogo et al., 2017). Relative humidity and wetness in the orchard should be controlled avoiding overhead irrigation (Llorente and Montesinos, 2006). Moreover, proper soil drainage is recommended as well as appropriated fertilization, avoiding both nutrient deficiencies or excess nitrogen that stimulates vegetative growth (Llorente and Montesinos, 2006). Other strategies include pruning the trees to promote the aeration, correct soil pH, keep the undergrowth low (< 0.20 m height), and maintain the orchard ground clear of weeds (Diogo et al., 2017). According with Reis et al. (2021), soil orchard management strategies can influence the infection since the overall incidence and prevalence of infection in ‘Rocha’ pear orchards were lower when the weed control system was mechanical cultivation compared with alleyways with natural cover.

8.4 Breeding

The identification of the gene controlling the susceptibility to *Stemphylium vesicarium* might be important for the development of resistant plants using breeding technologies. In a recent study, major QTL for susceptibility was identified (Cappai et al., 2018).

8.5 AgroEcological

Little is known about the effect of agroecological practices on the control of *S. vesicarium*, but a recent study (Arif *et al.* 2021) suggested that particular types of biochar could lead to disease suppression in onion.

9 References

Alberoni, G., Cavallini, D., Collina, M., Brunelli, A., 2010. Characterisation of the first Stemphylium vesicarium isolates resistant to strobilurins in Italian pear orchards. Eur. J. Plant Pathol. 126, 453–457.

Alberoni, G., Collina, M., Cavallini, D., Brunelli, A., 2008. Stemphylium vesicarium resistance to fungicides on pear in Italy. Mod. Fungic. Antifung. Compd. V 15th Int. Reinhardsbrunn Symp. Friedrichroda Ger. May 6-10 2007 167–172.

Alberoni, G., Collina, M., Pancaldi, D., Brunelli, A., 2005. Resistance to dicarboximide fungicides in stemphylium vesicarium of italian pear orchards. Eur. J. Plant Pathol. 113, 211–219.

Arzanlou, M., Khodaei, S., Babai-Ahari, A., 2012. Helianthus annuus as a natural host for Stemphylium vesicarium in Iran. Australas. Plant Dis. Notes 7, 167–170.

Aveling, T.A.S., Snyman, H.G., 1993. Infection studies of Stemphylium vesicarium on onion leaves. Mycol. Res. 97, 984–988.

CABI, 2006. Stemphylium vesicarium. [Distribution map]. Distrib. Maps Plant Dis.

Câmara, M., O'Neill, N., Berkum, P., 2002. Phylogeny of Stemphylium spp. Based on ITS and Glyceraldehyde-3-Phosphate Dehydrogenase Gene Sequences. Mycologia 94, 660–672.

Cappai, F., De Franceschi, P., Ciriani, A., Collina, M., Dondini, L., 2018. QTLs for susceptibility to Stemphylium vesicarium in pear. Mol. Breed. 38, 24.

Darrag, I.E., Zayed, M.A., El-Mosallamy, H.M., El-Gantiry, S.M., 1982. Studies on *Stemphylium* leaf spot of soybean in Egypt. Agric. Res. Rev. 60, 93–103.

Diogo, E., Sánchez, C., Leão, M., Sousa, R. de, 2017. A estenfiliose da pereira rocha em Portugal. Agrotec 24, 46–48.

Falloon, P.G., 1987. Etiology and Epidemiology of Stemphylium Leaf Spot and Purple Spot of Asparagus in California. Phytopathology 77, 407.

Gazzetti, K., Brunelli, A., Ciriani, A., Collina, M., 2015. Role of site-specific allele replacement into SvHK1 locus in the study of S. vesicarium resistance to dicarboximide and phenylpyrrole fungicides. In: H. B. Deising, B. Fraaije, A. Mehl, E. C. Oerke, H. Sierotski, G. Stammler (Eds.), Modern Fungicides and Antifungal Compounds VIII. DPG Verlag, Braunschweig, Germany, pp. 95–100.

Gazzetti, K., Diaconu, E.L., Nanni, I.M., Ciriani, A., Collina, M., 2019. Genome Sequence Resource for Stemphylium vesicarium, Causing Brown Spot Disease of Pear. Mol. Plant-Microbe Interactions® 32, 935–938.

Ichikawa, K., Sato, T., 1994. Leaf and Stem Spot of Aster Caused by Stemphylium vesicarium. Jpn. J. Phytopathol. 60, 523–526.

Isidoro, N., Azevedo, J., 2005. Capacidade do *Stemphylium* da flora espontânea provocar infecções em pereira Rocha. In: Manual Técnico de Produção Integrada Da Pêra Rocha. pp. 107–112.

Johnson, G., Sangchote, S., Cooke, A.W., 1990. Control of stem end rot (Dothiorella dominicana) and other postharvest diseases of mangoes (cv. Kensington Pride) during short-and long-term storage. Tropl Agric 67, 183–187.

Köhl, J., Groenenboom-de Haas, B., Goossen-van de Geijn, H., Speksnijder, A., Kastelein, P., de Hoog, S., Gerrits van den Ende, B., 2009a. Pathogenicity of Stemphylium vesicarium from different hosts causing brown spot in pear. Eur. J. Plant Pathol. 124, 151–162.

Köhl, J., Groenenboom-de Haas, B.H., Kastelein, P., Rossi, V., Waalwijk, C., 2009b. Quantitative detection of pear-pathogenic Stemphylium vesicarium in orchards. Phytopathology 99, 1377–1386.

Köhl, J., Jong, P., Kastelein, P., Haas, B., Anbergen, R., Balkhoven, H., Wubben, J., 2013. Dynamics of pear-pathogenic Stemphylium vesicarium in necrotic plant residues in Dutch pear orchards. Eur. J. Plant Pathol. 137, 609–619.

Koike, S.T., O'Neill, N., Wolf, J., Van Berkum, P., Daugovish, O., 2013. Stemphylium Leaf Spot of Parsley in California Caused by Stemphylium vesicarium. Plant Dis. 97, 315–322.

Lamprecht, S.C., Baxter, A., Thompson, A.H., 1984. *Stemphylium vesicarium* on *Medicago* spp. in South Africa. Phytophylactica 16, 73–75.
Llorente, I., Montesinos, E., 2002. Effect of Relative Humidity and Interrupted Wetness Periods on Brown Spot Severity of Pear Caused by Stemphylium vesicarium. Phytopathology® 92, 99–104.
Llorente, I., Montesinos, E., 2004. Development and Field Evaluation of a Model to Estimate the Maturity of Pseudothecia of *Pleospora allii* on Pear. Plant Dis. 88, 215–219.
Llorente, I., Montesinos, E., 2006. Brown Spot of Pear: An Emerging Disease of Economic Importance in Europe. Plant Dis. 90, 1368–1375.
Llorente, I., Moragrega, C., Ruz, L., Montesinos, E., 2012. An update on control of brown spot of pear. Trees Berl. Ger. West 26, 239–245.
Llorente, I., Vilardell, A., Montesinos, E., 2006. Infection Potential of Pleospora allii and Evaluation of Methods for Reduction of the Overwintering Inoculum of Brown Spot of Pear. Plant Dis. 90, 1511–1516.
Llorente, I., Vilardell, A., Vilardell, P., Pattori, E., Bugiani, R., Rossi, V., Montesinos, E., 2010. Control of brown spot of pear by reducing the overwintering inoculum through sanitation. Eur. J. Plant Pathol. 128, 127–141.
Llorente, I., Vilardell, P., Bugiani, R., Gherardi, I., Montesinos, E., 2000. Evaluation of BSPcast disease warning system in reduced fungicide use programs for management of brown spot of pear. Plant Dis. 84, 631–637.
Loebler, M., Sánchez, C., Muchagato Maurício, E., Diogo, E., Santos, M., Vasilenko, P., Cruz, A.S., Mendes, B., Gonçalves, M., Duarte, M.P., 2020. Potential Application of Propolis Extracts to Control the Growth of Stemphylium vesicarium in “Rocha” Pear. Appl. Sci. 10, 1990.
Montesinos, E., Bonaterra, A., 1996. Dose-response models in biological control of plant pathogens: an empirical verification. Phytopathology 86, 464–472.
Montesinos, E., Bonterra, A., Ophir, Y., Beer, S.V., 1996. Antagonism of selected bacterial strains to Stemphylium vesicarium and biological control of brown spot of pear under controlled environment conditions. Phytopathology 86, 856–863.
Montesinos, E., Moragrega, C., Llorente, I., Vilardell, P., 1995a. Susceptibility of Selected European Pear Cultivars to Infection by *Stemphylium vesicarium* and Influence of Leaf and Fruit Age. Plant Dis. 79, 471.
Montesinos, E., Moragrega, C., Llorente, I., Vilardell, P., Bonaterra, A., Ponti, I., Bugiani, R., Cavanni, P., Brunelli, A., 1995b. Development and Evaluation of an Infection Model for *Stemphylium vesicarium* on Pear Based on Temperature and Wetness Duration. Phytopathology 85, 586.
Montesinos, E., Vilardell, P., 1992. Evaluation of FAST as a forecasting system for scheduling fungicide sprays for control of Stemphylium vesicarium on pear. Plant Dis. USA.
Moragrega, C., Carmona, A., Llorente, I., 2021. Biocontrol of stemphylium vesicarium and pleospora allii on pear by bacillus subtilis and trichoderma spp.: Preventative and curative effects on inoculum production. Agronomy 11, 1455.
Olsen, K.J.K., Rossman, A., Andersen, B., 2018. Metabolite production by species of Stemphylium. Fungal Biol. 122, 172–181.
Ponti, I., Brunelli, A., Tosi, C., Basaglia, M., Bevilacqua, T., Emiliani, G., Cont, C., Viccinelli, R., 1993. Verifica dell’attività di diversi preparati contro la maculatura bruna del pero. Verifica Dellattività Diversi Prep. Contro Maculatura Bruna Pero 43, 45–52.
Ponti, I., Cavanni, P., Brunelli, A., 1982. Maculatura bruna delle pere: eziologia e difesa. Inf. Fitopatol. 32, 35–40.
Porta-Puglia, A., 1981. Stemphylium vesicarium (Wallr.) Simmons on tomato in the Marches. Ann. DellIstituto Sper. Patol. Veg. Roma 7, 39–46.

Prados-Ligero, A.M., González-Andújar, J.L., Melero-Vara, J.M., Basallote-Ureba, M.J., 1998. Development of Pleospora allii on Garlic Debris Infected by Stemphylium vesicarium. Eur. J. Plant Pathol. 104, 861–870.

Puig, M., Ruz, L., Montesinos, E., Moragrega, C., Llorente, I., 2015. Combined morphological and molecular approach for identification of <i>Stemphylium vesicarium</> inoculum in pear orchards. Fungal Biol. 119, 136–144.
Reis, P., Rego, C., Mota, M., Comporta, T., Oliveira, C.M., 2021. Brown spot disease in ‘Rocha’ pear Portuguese orchards. Acta Hortic. 335–342.
Rossi, V., Pattori, E., 2009. Inoculum reduction of Stemphylium vesicarium, the causal agent of brown spot of pear, through application of Trichoderma-based products. Biol. Control 49, 52–57.
Rossi, V., Pattori, E., Bugiani, R., 2008. Sources and seasonal dynamics of inoculum for brown spot disease of pear. Eur. J. Plant Pathol. 121, 147–159.
Rossi, V., Pattori, E., Giosué, S., Bugiani, R., 2005. Growth and sporulation of Stemphylium vesicarium, the causal agent of brown spot of pear, on herb plants of orchard lawns. Eur. J. Plant Pathol. 111, 361–370.
Sharma, S., Hay, F.S., Pethybridge, S.J., 2020. Genome Resource for Two Stemphylium vesicarium Isolates Causing Stemphylium Leaf Blight of Onion in New York. Mol. Plant-Microbe Interactions® 33, 562–564.
Simmons, E.G., 1969. Perfect States of Stemphylium. Mycologia 61, 1–26.
Singh, P., Bugiani, R., Cavanni, P., Nakajima, H., Kodama, M., Otani, H., Kohmoto, K., 1999. Purification and Biological Characterization of Host-Specific SV-Toxins from Stemphylium vesicarium Causing Brown Spot of European Pear. Phytopathology 89, 947–953.
Singh, P., Park, P., Bugiani, R., Cavanni, P., Nakajima, H., Kodama, M., Otani, H., Kohmoto, K., 2000. Effects of Host-Selective SV-Toxin from *Stemphylium vesicarium* , the Cause of Brown Spot of European Pear Plants, on Ultrastructure of Leaf Cells: Effects of *Stemphylium vesicarium* , the Cause of Brown Spot of European Pear. J. Phytopathol. 148, 87–93.
Sousa, A.J. t., Pina, A.M., Sousa, A.L., 2004. A Stemphyliose da pereira Rocha em Portugal, Perspectivas para o seu controlo, Cadernos Rurais. AVAPI.
Stricker, S.M., Gossen, B.D., McDonald, M.R., 2021. Risk assessment of secondary metabolites produced by fungi in the genus Stemphylium. Can. J. Microbiol. 67, 445–450.
Woudenberg, J.H.C., Hanse, B., van Leeuwen, G., Groenewald, J.Z., Crous, P., 2017. Stemphylium revisited. Stud. Mycol. 87.
Zapata-Sarmiento, D.H., Palacios-Pala, E.F., Rodríguez-Hernández, A.A., Medina Melchor, D.L., Rodríguez-Monroy, M., Sepúlveda-Jiménez, G., 2020. Trichoderma asperellum, a potential biological control agent of Stemphylium vesicarium, on onion (Allium cepa L.). Biol. Control 140, 104105.