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THE MAIN FUNGAL DISEASES IN STRAWBERRIES CROP - REVIEW

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Abstract

Strawberry cultivation is one of the most important fruit crops in the world from a commercial point of view, but it is constantly threatened by fungal pathogens, which cause significant economic losses. The decline of strawberry plants is also seen as a severe threat to strawberry production in Romania. Due to recent restrictions on pesticide use and the lack of effective and efficient alternatives, achieving consistently high yields has become difficult. The most important diseases that cause significant economic losses are: Botrytis cinerea, Phytophthora cactorum, Colletotrichum fragariae. They affect all parts of the plant: flowers, fruits, leaves and roots causing significant annual losses: 40% for Phytophthora cactorum attack, 50% for Colletotrichum fragariae and up to 80% for Botrytis cinerea attack. This review provides an overview of the latest studies on the main fungal pathogens, the measures and control strategies currently available, the risk factors influencing the development of pathogens and what strategies we can address to limit the economic losses of the strawberry crop.

Key words*: strawberries, pathogens, control, strategies.*

INTRODUCTION

The strawberry cultivated with large fruits (*Fragaria* × *ananassa* Duchesne) has its origins in Europe in the 18th century. Cultivated strawberry (*Fragaria x ananassa* Duchesne), is a hybrid of two mostly dioecious octoploid species, *Fragaria chelonesis* Duchesne and *Fragaria virginiana* Duchesne. It is a short-lived perennial herb that grows predominantly in temperate climates. Its fruits are a rich source of vitamins and minerals (Sharma et. al., 2009).

Most strawberries (*Fragaria x ananassa* Duchesne) in Romania are produced either in the south of the country (the area with the highest strawberry production being Hotarele in Giurgiu County) or in the North-West (Satu-Mare). Fresh fruit is usually shipped to cities and areas where soil or temperatures do not answer to crop requirements. Therefore, Romanian growers need varieties that produce attractive and aromatic fruits and in order to reach these qualities, the appearance of pathogens in the culture must be avoided. Crops and fruit quality are usually reduced when strawberry plants are infected with fungi. In the last ten years, the pathogens that cause significant losses in culture are: Gray mould (*Botrytis cinerea*), Collar rot of apple (*Phytophthora cactorum*) and Anthracnose (*Colletotrichum fragariae*).

STRAWBERRIES PATHOGENS

1. Gray mould (*Botrytis cinerea*)

Gray mould is a necrophytic pathogen that causes significant damage to strawberry crops. *Botrytis* fruit rot, commonly known as gray mould, is caused by the fungus *Botrytis cinerea* and is one of the most serious strawberry pathogens in Romania and across the world. The fungus affects all aerial sections of the plant. The majority of economic damage occurs when flowers and fruit are damaged, resulting in yield losses of up to 80% in some cases. *Botrytis cinerea* is also a post-harvest pathogen because infections that start in the field continue to grow during storage and transportation at refrigeration temperatures (Mertely et al., 2018).

Symptoms. The manner and timing of infection differ based on the plant type and plant parts infected. *Botrytis* disease symptoms vary widely depending on the host and plant section affected. A grey to brown discolouration, water soaking, and fuzzy white grey to brown mould (mycelium and conidia) forming on the surface of damaged tissue and

isolated lesions are symptoms. The pathogen can infect fruits through the bloom persisting in the very early stages of growth and developing just after harvest when the fruit is fully ripe. This method of infection is significant in strawberries and is thought to be the primary cause of deterioration in ripe fruit after harvest. Blossom blights frequently precede and contribute to fruit and stem end rots. The fungus frequently spreads from the fading petals across the inflorescence and develops on the fruit, causing blossom-end rot. Conidia can infect the fruit directly through growth cracks, cut stem scars, insect wounds, or lesions caused by other pathogens. Infected fruits develop water-soaked, yellowish green or greyish brown irregular lesions that might be mushy and spongy in texture (Elad et al., 2007).

The host. In Romania, the majority of strawberries are May-bearing varieties cultivated as perennials in matted rows for 2-5 years. Strawberries are typically mulched with wheat straw in the fall to give winter protection, and the straw is removed or placed between the rows in the spring. Plant regrowth occurs in April, followed by overlapping blooming and fruit development periods in May and June. The crop is harvested between mid-May and mid-June and then restored. Renovation may include leaf mowing, tilling between rows, row narrowing, plant thinning and fertilizer and pesticide application.

Gray mould also attacks other crop plants, being identified by the anamorphic form of *Botrytis cinerea* (Cristea, 2005; Docea et al., 2012; Gheorghies & Cristea, 2001; Docea E. & Cristea Stelica, 2003).

Infection and colonization. *Botrytis cinerea* can infect strawberry leaves, flowers, fruits, and crowns. Infected tissues are usually asymptomatic, and colonization does not progress until the tissues senesce, ripen, or die, but lesions can form on the calyces, petals, stamens, or green receptacles.

The interactions between *Botrytis cinerea* occurrences are crucially dependent on strawberry leaves. Although the pathogen does not cause symptoms on the foliage, it can be recovered from surface sterilized infected green leaves and it frequently sporulates on the leaves immediately after death. Recent discoveries have put a spotlight on some of the previously enigmatic relationships between leaf growth and infection and colonization. The receptivity of field grown plant leaves to *Botrytis cinerea* it was found to be high for developing leaves, zero for mature leaves and low for senescent leaves.

Infection of the flowers by *Botrytis cinerea* conidia is a crucial precursor to the attack on the fruit. Senescent sepals, petals, and stamens are key sources of mycelium capable of penetrating strawberry fruit, debunking the commonly held belief that the fruit is generally infected directly by conidia. Just 1% of fruit infections to conidia, with the vast majority attributed to mycelium in detached petals, decomposing berries, and receptacles sticking to the fruit, or in diseased floral portions that remained connected to the receptacles. Conidia germinated easily in stigmatic fluid and infected stigmas in the absence of dew or other extra water, but it took 4-6 weeks to develop along the styles, making fruit invasion an implausible method. On the other hand, the infection frequently develops along the filaments of infected stamens and into the receptacle. Mycelium growth into green receptacles from diverse floral parts is fast until after the fruit ripens, giving rise to the fruit's distinctive stem-end rot (Sutton & John, 1990).

Grey mould in strawberries can be caused by *B. cinerea* infections of open flowers (primary infections) or by penetration of fruit receptacle tissues (secondary infections). *B. cinerea* infects flower organs during or just after flowering, causing hyphae to develop into the receptacle. Primary inoculum sources include overwintering sclerotia as well as conidia or mycelium from infected neighboring plants. Primary infections in fruit can be facilitated by infected senescent petals, stamens, and calyxes. Despite the fact that styles are commonly infected, histological investigations demonstrate that fungal development appears to be highly prevented and never reaches the receptacle. Fungal development in colonized stamens, on the other hand, can reach the receptacle in some cultivars (Bristow et al., 1986).

Following *B. cinerea* infection of the unripe receptacle, fungal growth normally stops and a symptomless quiescent phase ensues. The processes that because dormant infections are

yet unknown. Proanthocyanins (PAs) appear to promote *B. cinerea* quiescence in unripe fruit by inhibiting the activity of fungal enzymes such as polygalacturonases (PGs), which are required for aggressive host infection (50% inhibition in unripe fruit compared to 8% inhibition in ripe fruit). Despite the fact that the PA concentration of the fruit stays constant during ripening, increased polymerization of PAs results in reduced inhibitory action in mature fruit. Similarly, anthocyanins may postpone or promote *B. cinerea* infections. Strawberries exposed to white fluorescent light, had higher anthocyanin levels and delayed the growth of grey mold (van Baarlen et al. 2007).

The fungus begins the necrotrophic phase without quiescence during secondary infections. Conidia for secondary infections can be found in a variety of places, including senescent leaves and infected fruit. Secondary inoculum is mostly obtained from conidia found in *B. cinerea*-infected floral sections. It is believed that organic pieces that come into touch with the fruit, such as petals and stamens, cause more than 64% of strawberry infections. Secondary infections can also occur as a result of nesting, which is characterized by direct penetration of mycelia growing on neighboring plant parts such as diseased leaves and fruit. Secondary infections, in general, progress quickly, and *B. cinerea* can complete germination and infection as quickly as 16 hours after inoculation (Jarvis, 1962). Because of the availability of high-quality reference genome sequences, the infection mechanisms of *B. cinerea* have been examined in model organisms and further defined. By using a range of virulence mechanisms, the fungus is known to actively enhance plant sensitivity. *B. cinerea* uses sRNAs and effector proteins to prevent premature host cell death and immunological responses in the early stages, allowing the fungus to establish itself inside the host and amass biomass prior to the necrotrophic phase (Veloso & Van Kan, 2018). *B. cinerea* Dicer-like proteins DCL1 and DCL2 have been shown to create sRNAs that are released from fungal hyphae and translocated to the plant cell, where they interact with host RNAi processes to silence host immune response genes in arabidopsis and tomato leaves (Weiberg et. al., 2013).

Detection and Quantification. Late stage infections are easily identified in symptomatic material by the emergence of grey/brown conidial clusters on infected material's surfaces. It is difficult to detect infections in non-symptomatic material, such as in the early stages of infection or latent and endophytic infections. Surface sterilization, followed by freezing of tissues or berries for 2 hours at 12°C or dipping in 300 g/ml paraquat, followed by air drying and incubation in a humid atmosphere on wet filter paper or selective media for 7-10 days, has been widely used (Fillinger & Elad, 2016).

Using GC-MS, Van den Driessche et al. (2012) identified a number of volatile biomarkers in strawberries infected with *B. cinerea*. They demonstrated that while these markers may be used to track infections, they cannot detect pre-symptomatic infections. If the sensitivity of such non-destructive procedures could be enhanced, they may be extremely valuable in the food sector. Semi-selective and differential media are based on the selective suppression of competing bacteria, the promotion of target organism development, and/or the expression of a *Botrytis*-specific trait. Viable conidia germinate and form colonies on a variety of media in the presence of free water, nitrogen, and phosphate, although they do not always sporulate. A microscopic investigation is required to confirm the development of any *Botrytis* species (Dewey, 2000). A variety of immunoassays have been applied to detect and to a lesser degree quantify, *Botrytis* infections in plants. Enzyme-linked immunosorbent tests, particularly plate trapped antigen-immunosorbent assays, are by far the most prevalent (PTA-ELISAs). Ricker et al. (1991) showed that PTA-ELISAs may be used to assess levels of *Botrytis* antigens in juice from diseased grapes using rabbit antisera. Recently, the same approach was used to identify and quantify *B. cinerea* in grape juice, wines, pear stems, and strawberries using the genus-specific monoclonal antibody BC-12.CA4 (Dewey, 2000).

Life cycle. Gray mold typically begins in contaminated plant debris from previous crops that has been left in the field. When temperatures rise, such as in early spring, the

mycelium in the debris begins to grow. Mycelium begins to form structures known as conidiophores when exposed to bright light. Conidiophores produce spores named condias, which are subsequently dispersed via the air and can come into touch with crop leaves or stems. They germinate and begin to attack there. For the spores to be released from the conidiophores, there must be a quick drop in humidity and an increase in temperature. This is frequently the case in the early hours of the morning. Raindrops splashing on an infected plant can also aid in the spread of the spores. Insects are another mode of transmission for conidia from one infected plant to another, and they are a major source of infection. Infected crops and gardens in the area might also be a source of infection.

Moisture and nutrients must be present on or near the plant for the spore to germinate. Moisture can occur through condensation on the plants, which is generated by air humidity levels of more than 95%, or from any plant sap that escapes as a consequence of damage to the plant's exterior surface. Germ tubes emerge from the spore when it germinates. An appressoria at the end of these tubes generates an infection peg that penetrates plant tissue. The peg does not penetrate the plant tissue immediately. It must initially produce enzymes that contribute in the elimination of the plant's first cellular barrier (Williamson et al., 2007).

Because the cuticle of healthy tissue is frequently quite strong, the fungus has a better chance of penetrating damaged, weak, or senescent tissue. Stomata and wounds can potentially be entry points for infection. That is why *Botrytis* commonly appears after a caterpillar attack, because the fungus will use the damage created by the insect bites to penetrate the plant. *Botrytis* secretes proteins and phytotoxic chemicals that cause the collapse and death of cells close to the host. The plants immune system also influences the rate at which infection occurs. The plants defenses are occasionally weaker in the autumn, allowing the half-dormant *Botrytis* to do serious damage (AbuQamar et al., 2016). Fungal spores germinate and spread by wind and/or water all across the spring when the weather is cold and rainy. Heavy rain or overhead irrigation, along with cold temperatures, promotes *B. cinerea* development. When strawberries or flowers stay wet for 24 hours or more, the infection risk reaches 90%. The pathogen that causes fruit infection is mostly induced by blossom infection, however the disease can remain dormant until the fruit ripens (Mattson & Daughtrey, 2019).

Ecology. *Botrytis cinerea*, like many fungus, is only harmful to a host when particular environmental conditions are satisfied. If these conditions are not satisfied, the fungus will generally remain a saprophyte inside the crop, feeding on senescing or dead tissue. *Botrytis cinerea* may be present on all continents, but because to the 9 distribution of economic hosts, the disease it causes are mostly linked with cool temperate and warm-temperate zones (Mehli et. al., 2005).

Management. Despite significant efforts, strawberry resistance to gray rot has yet to be improved, and the mechanisms of tolerance to the pathogen *Botrytis cinerea* attack are still poorly known and investigated.

Currently, strawberry gray rot control is focused on cultural, chemical and biological approaches. As a result, the yellowed, discolored, and dried leaves will be removed in the spring. Mulching is done with clean straw to minimize fruit contact with the soil and soiling during the wet season. To avoid crushing the seedlings or the leaves, the transit through the culture should be done with extreme caution. Plants that are susceptible to botrytis (lettuce, tomatoes) should be avoided in close proximity to strawberries. Chemical protection begins when the inflorescences are spread. *B. cinerea* infections that cause postharvest decay typically emerge before harvest, in the field, and can stay latent until fruit storage. Because there are no infection symptoms and no reliable ways for predicting the risk of this disease, preventative measures should be taken before symptoms appear. Field applications of synthetic fungicides during the crop growth cycle have traditionally been used to reduce gray mold infestation. The fungicides are administered around strawberry plant flowering and are repeated till harvest, depending on the weather and the preharvest

period for the different formulations (Feliziani & Romanazzi, 2013).

Botrytis cinerea is a classic "high-risk" pathogen in terms of fungicide resistance, therefore chemical treatment of gray rot is a constant issue, with new fungicides prone to resistance.

With the development of new fungicides, *Botrytis cinerea* control has become increasingly successful. Over the last four decades, a standard practice has emerged in which frequent chemical management has become a must. Fungicide programs remain the foundation of *Botrytis cinerea* control, although they have lately become less successful and increasingly unpopular with the public. Environmental authorities and consumers in many nations are worried about pesticide usage in agriculture, and as a result, there is a growing demand for organically farmed foods. This environmental issue has sparked much study and the development of alternate approaches or systems for controlling *Botrytis cinerea* in strawberries.

In the struggle against gray rot, biological control is an excellent option. Biological control agents are known to be mostly bacteria and yeast that are "antagonistic" to the pathogens that cause postharvest strawberry fruit deterioration. They compete with the pathogens for nutrients and space, or they parasitize the pathogens by creating antibiosis pressures, such as the production of volatile compounds that are noxious or unpleasant to the pathogens, or they can induce resistance in the host tissue to strengthen the plant's defenses against the pathogens. Numerous experiments have been conducted in recent years to isolate and test microorganisms that can fight strawberry infections (Jamalizadeh et. al., 2011).

Several microorganism-based products are already registered against *B. cinerea*, and not just for strawberry. *Pseudomonas syringae* (BioSave; JET Harvest Solutions, Longwood, FL, USA), *Bacillus subtilis* (Serenade; Bayer, Leverkusen, Germany), *Candida sake* (Candifruit; IRTA, Lleida, Spain) and *Metschnikowia fructicola* (Shemer; Bayer, Leverkusen, Germany) are some of the most well-known commercially available biofungicides. Inorganic salts have been proven to be active antimicrobial agents against a variety of phytopathogenic fungi, with bicarbonates being offered as a safe and effective alternative way of controlling postharvest rot of fruits and vegetables, including strawberry fruit. In addition to being non-toxic and having a low environmental impact at their effective quantities, these salts are affordable. Calcium-rich formulations can be used to fortify the central lamellae of strawberry fruit cells, increasing their resilience to mechanical and biological harm (Karabulut et. al., 2004). A modified storage atmosphere with carbon dioxide enrichment, to reduce the incidence and severity of decay and thus to extend the postharvest life of strawberry fruit, is one of the most commonly used postharvest treatments to control fungal growth and reduce the respiration rate of strawberry fruit. However, negative impacts on color and flavor have been recorded in certain cases following exposure to extremely low oxygen and extremely high carbon dioxide levels. Very high oxygen conditions have been studied as an alternative to standard modified environment packing of fruits and vegetables, including strawberry fruit, since very high oxygen inhibits grey mould growth and can avoid unwanted anoxic fermentation (Allende et al., 2007). Among the different techniques of reducing plant gray rot, the use of plant extracts is an option, characterized by: lack of toxicity to humans and the environment, selectivity, biodegradability, and a high chemical diversity, having a wide range of metabolites. Secondary, many of them have not yet been explored in connection to pesticidal efficacy. *Capsicum* plant extracts such as *Capsicum anuum* (pepper), *Capsicum chinense* (habanero pepper), and *Capsicum frutescens* (hot pepper) have demonstrated in vitro, antifungal efficacy against the pathogen *B. cinerea*, eventually limiting spore germination (Wilson et al., 1997).

Cloves (*Syzygium aromaticum*) include natural chemicals that have antifungal activity against gray rot. In addition, the water and ethanol garlic extract (in vitro) acts as an antifungal by totally suppressing mycelial development and spore germination of *Botrytis cinerea* (Šernaitė et al., 2020). Although the volume of experimental research on the antibotritic action

of various plant extracts is remarkable, quite a few conditioned products based on such extracts have been delivered for agricultural practice.
These include BM-608 products, based on essential oils from *Melaleuca alternifolia* (Adebayo et al., 2013) or *Gloves Off*® (commercial disinfectant produced by Planet People and Laboratoire M2, INC, Sherbrooke, QC, Canada) from oils from *Thymus* (Gebel & Magurno, 2014). This direction of investigation and development being open to future research.

Biocontrol Tests. To be effective, microorganisms introduced into a crop to manage a disease must be able to interact effectively with the pathogen, the host, and other organisms within the prevailing microclimatic circumstances. The biocontrol system is very dynamic, including host growth and development, pathogen infection cycles and serial dispersals, quantitative alterations in biocontrol agent and indigenous organism populations, and microclimatic oscillations. In general, epidemics cannot be well reproduced in the laboratory, growth room, or greenhouse, and biocontrol experiments performed in these settings should be interpreted accordingly. Under regulated settings, biocontrol testing can be used efficiently for preliminary screening of organisms and for supplementing agricultural operations.
Gliocladium roseum provides a number of benefits for effective biocontrol of *Botrytis cinerea* in strawberries. Because it is effective in leaves, flowers, and fruits, it can successfully be targeted against *Botrytis cinerea* at the inoculum source in the leaves or to directly protect the flowers and fruits. The ability of *G. roseum* to penetrate and survive in the leaves results in extraordinary antagonist persistence in the foliage for weeks or months after application, in contrast to many biocontrol agents that are active against pathogens mainly on the phylloplane and rapidly decline in number and activity after application.
G. roseum, when administered once when the leaves are green, has the ability to suppress *Botrytis cinerea* in each leaf flush due to its persistence in green leaves and biocontrol action after the leaves senesce and die. In contrast to chlorothalonil, *G. roseum* is less effective or ineffective when applied to senescing or dead leaves, according to field studies. *G. roseum* inoculum generation can easily scaled up on low-cost substrates like as wheat grains, which may improve the cost-effectiveness of biocontrol (Sutton, 1995).

2. Collar rot of apple (*Phytophthora cactorum*)

In strawberry crop, *Phytophthora cactorum* is one of the most damaging pathogens. It is a disease that has spread widely in Western Europe and the United States, destroying, in some years, large areas cultivated with strawberries or reducing production in infected plots. It is a specific root disease, but it attacks all the organs of the plant resulting in yield losses of up to 40% in some cases.
Phytophthora cactorum is a polyphagic organism found in temperate region soils. It causes disease in 200 crop species from 60 families. This fungus primarily affects ornamental plants (such as rhododendron, pansy, pelargonium, and begonia), fruit shrubs and trees (such as gooseberry, currant, strawberries, peach, raspberry, apple tree, and cherry) and forest trees (such as birch, black alder, common beech, common ash, and spruce) causing phytophthorosis (Hantula et al., 2000).
The remains of infected plants left over from previous plantings, water used for irrigation from neighboring ponds or rivers, irrigation systems, contaminated soil, or infected seedlings may be the source of *P. cactorum* on plantations. Oospores of *P. cactorum* are thick-walled spores and they may survive in soil for up to 6 years (Orlikowski et al., 2017).
Strawberry is resistant to the majority of *P. cactorum* strains, with just a few specialist *P. cactorum* isolates capable of causing crown rot. On the other hand, many popular strawberry cultivars are vulnerable to specialist *P. cactorum* crown rot isolates, and just a few cultivars regularly show resistance. According to Shaw et al., (2008) resistance to the crown rot pathotype is a polygenic characteristic. Aside from the genetic background, the physiological condition of the plant is important: cold-stored and/or damaged plants are especially sensitive to the disease, and

young plants are more susceptible than older ones.

Symptoms. Disease outbreaks frequently begin with isolated areas of diseased strawberry plants. They grow in size, especially down slopes where water dispersion may fast cause extensive regions to be impacted. Symptoms can appear on the roots as early as late autumn, but they usually do not appear on the above-ground sections of the plants until late spring or early summer, when it can be difficult to uncover confirming evidence of the pathogen in the roots.Symptoms usually appear on the upper parts of plants that come under stress in late spring or early summer, especially in low-lying, wet areas. Plants frequently do not develop or grow in a stunted manner. They may collapse shortly before ripening or yield only a few small fruits. Younger leaves might be blue-green, whereas elder leaves can be yellow or red. Digging up the plants shows a rotting and underdeveloped root system.

Lateral feeder roots are typically rotten and destroyed by the time plants are dug. The adventitious roots decay from the tips upwards and frequently have a grey to brown coloration at their distal ends. Cutting apart the top, white, unrotted sections of such roots reveals steles that range in color from wine-red to brick-red, thus the term red core. In extremely sensitive varieties, the color can spread fairly far beyond the rotten regions of the roots, all the way into the crown.

Phytophthora cactorum may survive in soil as sexual oospores that germinate under moist circumstances to develop mycelium and sporangia, which release motile asexual zoospores that infect the plant collar and fruits. The infection appears as dark staining inside the collar, and subsequent wilting leads to the plant's death (Poimala et al., 2021).

Infection. Several Phytophthora species are classified as hemibiotrophs because they retain a biotrophic interaction with their host for a period of their life cycle. Prior to penetration of a host cell, sporangia, zoospores, cysts, and germinating cysts with germ tubes are generated, all of which are necessary for plant infection and disease development. Infection by root pathogenic oomycetes like as P. cactorum begins with the production of motile, wallless zoospores, which encyst on host surfaces after a chemotactic stage before host penetration occurs. These pre-infection structures are expected to be rich in chemicals important in infection establishment and elicitation of plant defenses (Xiaoren et al., 2011).

Wet conditions promote infection. Oospores in the soil and infected transplants are the principal sources of inoculum. Once introduced, the disease can live for many years on certain farms, whilst other farms do not have recurring issues year after year. Oospores generate zoospores, which typically infect stolon stumps, rhizomes, or freshly cut runners through wounds. Frigo plants, or plants that have been stored in the cold, are highly sensitive to crown rot. The manifestation of disease is regulated by the time of planting as well as environmental circumstances. Because the fungus demands warm temperatures and sustained wetness, early plant collapse can begin within one month of sowing in the fall, with subsequent plant collapse occurring the following spring when regrowth starts and especially as fruit develops. Water allows the virus to travel (Pettitt & Pegg, 1994).

P. cactorum may persist in soil for extended periods of time at the water content and temperatures that are typical throughout the growth season. They sustain viability even when soil is held at - 10°C for several hours, indicating that they are evolved for long-term survival in the soil environment. While oospores may certainly live in soil for longer periods of time than sporangia due to their form and nature, sporangia must be considered as a more essential short-term inoculum unit. Sporangia are formed when oospores germinate. Sporangia can germinate directly and possibly infect a host, or they can create new sporangia at the ends of the germ tubes, or they can discharge zoospores under conditions favorable for indirect germination (Erwin & McCormick, 1971). Zoospores are the most likely to cause infections, and a recent study shown that they may survive in soil for several weeks. Sporangia and oospores both germinate in soil with a water content less than its field capacity (McIntosh, 1972).

Because of their fast lysis, mycelium and hyphal fragments appear negligible as inoculum or in pathogen persistence in soil.

Except in wet soil as sporangiophores sprouting from the tissue, it is unusual that hyphae grow to any degree on the surface of infected root tissue (Sneh & McIntosh, 1974).

The ideal temperature for germination is 10-15°C, however it can happen at 20°C as well as very slowly at 5°C. Temperatures between 10 and 17°C are ideal for infection. Infection can develop at temperatures as low as 2°C but not at temperatures as high as 25°C. It moves more slowly below 10°C, but more secondary inoculum is produced over longer periods of time, explaining why the disease is more severe following a rainy winter (Liu et al., 2018).

Methods used to determine levels of infection

Strawberry crown rot induced by *P. cactorum* is a difficult subject to quantify. Previous studies measured disease, pathogen virulence, and host resistance in terms of percentage mortality or the period of manifestation of the first symptoms.

Duncan (1985) isolated *P. fragariae* oospores from infected strawberry root tissues by comminution, followed by filtration and thorough washing, primarily for germination experiments. Harris (1985) retrieved *P. syringa* oospores in fallen apple leaves by comminution and selective sifting, followed by haemocytometer counts. Although these approaches are useful for estimating the inoculum potential in affected tissues, they do not provide a direct estimate of the amount of invading mycelium. The chemical measurement of chitosan is another method for determining the mycelial concentration of diseased plant tissues. However, due to the lack of chitin in the hyphal walls of this species, such chemical techniques cannot differentiate between living and non-viable mycelium and would be unsuitable for *Phytophthora* spp. (Bartniki-Garcia, 1966). Another method for measuring pathogen quantity is to apply an enzyme-linked immunosorbent test, which has been effectively used to identify *P. infestans* mycelium in potato leaf tissue (Harrison et al., 1990).

Detection of *P. cactorum*. Culture-based isolation methods are still widely used for detecting *P. cactorum* in strawberries, but with proper optimization and validation, PCR may offer significant benefits over culturing and other methods for monitoring this pathogen. Culture-based detection of *Phytophthora* species takes about a week and specific mycological expertise, whereas PCR-based detection, including DNA extraction, takes around 2-3 days and general molecular biology knowledge. There are enzyme-linked immunological assays available for detecting *Phytophthora* spp., however they are not species-specific (Olsson, 1995).

Diagnostic PCR techniques and primers for *Phytophthora* species that infect strawberries, such as *P. cactorum* and *P. fragariae* var. *fragariae*, have been developed. The nested PCR primers ADF1 and ADR1 were found to amplify a 520-bp DNA fragment from the ITS sections of the *P. cactorum* rRNA gene in strawberry root samples. PC1/PC2, developed as SCAR markers from a particular random amplified polymorphic DNA fragment to produce a PCR product of roughly 450 bp, were shown to be specific, sensitive, and robust for identifying *P. cactorum* in different host plants, including strawberry. Primers Ycac1F and Ycac2R, which amplify a 192-bp segment of the ras-related protein gene Ypt1, were recently shown to be specific for *P. cactorum* (Bhat & Browne, 2010).

Management. In agricultural fields, soil fumigation and good cultural methods offer effective *Phytophthora* management. Using certified seedlings, avoiding poorly drained soils, and preparing fields to allow sufficient soil drainage during wet weather are all examples of appropriate cultural practices. Because *Phytophthora* can be spread by water that has been drained from contaminated fields, avoid utilizing runoff water for irrigation or watering down field roadways for dust management. Even with tolerant cultivars, it is critical to adhere to excellent cultural practices (Koike & Gordon, 2018).

Mefenoxam (Ridomil Gold) and metalaxyl (different formulations) have been shown to be beneficial when applied by drip irrigation. These fungicides are most effective when sprayed in the fall, shortly after planting and after all overhead watering for plant establishment has been completed. In most

circumstances, an early spring spray is also beneficial as new plant development begins to accelerate. The time of the fall and spring seasons coincides with strong root development. Additional treatments can be done in areas with significant disease pressure in the spring (Frank et. al., 2019).

Chemical compounds are commonly utilized to minimize *P. cactorum* infection, despite the fact that this species and other *Phytophthora* spp. have showed the potential to develop resistance to such chemicals. *P. cactorum* has been shown to be resistant to dimethomorph, metalaxyl (mefenoxam), cymoxanil, and mancozeb. Resistance can develop quickly in certain populations, the frequency of *P. cactorum* strains resistant to metalaxyl reached up to 80% as early as four years after metalaxyl was first used in strawberry plant protection (Utkhede & Gupta, 1998).

Copper-based chemicals (fungicide group M1), such as the Bordeaux combination, have been used for a long time and are still effective. Copper hydroxide, copper oxide, basic copper sulfate, copper oxychloride, and copper ammonium carbonate are some other copper-based protectant fungicides. The Cu++ ion is the active agent against *Phytophthora* in each case. Some of these may leave residues on the leaf of plants. They are most commonly used during the dormant season. Acidic situations, such as tank mixing with phosphorous acids, will release an excessive amount of copper ions, causing plant damage (Hoitink & Powell, 1990).

Although the use of fungicidal chemicals remains an efficient technique of protecting plants in both systemic and eradicant applications, the possibility of resistance to such compounds has prompted research to identify alternate methods of plant protection that do not offer such a risk. Other ways of plant protection are being studied, frequently based on live biological control agents (BCAs) or their metabolites, in addition to the use of proven antiresistant approaches, which reduce the rate of resistance formation in the practical use of fungicides.

Fungi of the genus *Trichoderma*, *Aureobasidium pullulans* (yeast fungus), *Bacillus*, namely *B. amylolyquefaciens, B. megaterium, B. subtilis, B. velezensis, B. licheniformis*, and *B. cereus, Pseudomonas*, specifically *P. fluorescens* and *P. syringa* and the use of arbuscular mycorrhizal fungi from the genera *Glomus* and *Claroideoglomus* proved effective against *P. cactorum* (Pánek et al., 2021).

The very promising effect of *Trichoderma* spp. and solarization against *P. cactorum*, as well as cultural practices to reduce or eliminate standing water, the ability of the biocontrol agent to proliferate in field soil, and the increase in marketable yield, suggest that there may be future alternatives to traditional chemicals for strawberry disease control (Porras et al., 2007).

Some fertilization programs have been applied to control *Phytophthora* spp. They include using organic materials that release ammonia and nitrous acid, using sulfur-based fertilizers and amendments that reduce pH to less than 4 for acid-tolerant plants, reducing pH to less than 5 in high-aluminum soils (for plants with a tolerance for aluminum), applying foliar nutrients to compensate for rotting fibrous roots' loss of uptake, and avoiding excessive nitrogen fertilization, which makes the resulting succulent foliage more susceptible (Pscheidt & Ocamb, 2015).

Nursery transplants subjected to aerated steam in a closed chamber at 37°C for 1 hour followed by 44°C for 4 hours were substantially less likely to die from *Phytophthora crown rot*, according to studies conducted at the UF/IFAS GCREC. As a result, thermotherapy of transplants may be a viable option for nurseries in managing *Phytophthora* populations in plant stock (Natalia & Juliana, 2019).

3. Anthracnose (*Colletotrichum fragariae*)

Strawberry anthracnose is a serious disease that affects all parts of the plant, including the fruit, crowns, leaves, petioles, and runners. *Colletotrichum acutatum, C. gloeosporioides*, and *C. fragariae*, three related species of the fungus *Colletotrichum*, have been correlated to anthracnose. However, *C. acutatum* is the primary pathogen involved with the anthracnose fruit rot phase resulting in yield losses of up to 50% in some cases (Gunnell & Gubler, 1992).

Symptoms. Lesions of anthracnose fruit rot develop as dark, sunken lesions on contaminated fruit. Anthracnose lesions on green fruit are small, firm, deep, and dark brown or black. Ripening fruit lesions are bigger, harder, deep, and brown to dark brown. When it rains, the lesions get covered with a sticky, light orange liquid consisting of millions of spores (conidia) in a viscous liquid structure. Numerous lesions almost cover the fruit when conditions are favourable for infection, and lesions may form on petioles. When strawberry flowers become infected, they turn brown and remain attached to the plant. Flowers infected with the gray mold fungus *Botrytis cinerea* may present symptoms similar to those described above. Flower infections can also cause small dark spots on green button-sized fruit (Mertely et al., 2012).

Disease development. Despite various species of fungus in the genus *Colletotrichum* can produce anthracnose, *Colletotrichum acutatum* is the most prevalent species causing fruit rot in Romania. According to recent study, the fungus may grow and develop spores on the surface of seemingly healthy leaves.

Once the infection has established in the field, the fungus may survive in winter on affected plants and plant debris, such as dead leaves and mummified fruit. Warm, humid temperatures and rain promote spore formation and germination, and strawberry fruit infection. Spores are abundant on previously infected plant debris in the spring and early summer. Splashing rain, wind-driven rain, and people or equipment moving through the field all spread the spores. Because they are not airborne, they do not go a long distance in the wind. Spores need free water on the plant's surface to germinate and infect.

Temperatures between 25 and 30°C are ideal for infection on both immature and mature fruit. On infected fruit, the fungus generates secondary spores under favorable conditions. Rain spreads these spores, resulting in secondary infections throughout the growing season. Disease development can proceed at a rapid speed. Within a week or less, up to 90% of the fruit can become infected. Infection can occur in both immature and mature fruit; however, the disease is most prevalent in ripening fruit (Michael et al., 2016).

The host. The fruit rot produced by *C. acutatum*, which also infects many other fruit and vegetable crops such as apples, tomatoes, peppers, peaches, blueberries, blackberries, and grapes, causes the most economic losses on strawberries crops. The pathogen has been found on strawberries in practically every region of the world where they are farmed. Infected plants often do not flourish following transplantation and provide few berries at harvest (Smith, 2008).

Conidia germinate by creating sessile appressoria or germ tubes and terminal appressoria. Appressoria melanize and create pores through which the host can enter. There are two types of infections that can occur: intracellular hemibiotrophic infections, in which infection vesicles or swollen hyphae form within epidermal cells, and subcuticular, intramural necrotrophic infections, in which cuticular penetration is followed by hyphal proliferation within the cuticle and epidermal cell walls, but without penetration of the cell lumen. Both types of infections may lie dormant for a long time before substantial cell loss, colonization, and symptom development occur (Leandro et al., 2001). Without host plants, *Colletotrichum acutatum* may persist in soil for at least 9 months (Bolda et al., 2018).

Curry et al. (2002) used light and electron microscopy to investigate the infection of strawberry petioles and stolons by *C. acutatum* and *C. fragariae*. Both fungal species entered the host tissue similarly; however, *C. fragariae* infiltrated the plants faster than *C. acutatum*. Both species used an appressorium to enter the cuticle, and their hyphae developed within the cuticle and cell walls of epidermal, subepidermal, and subtending cells. They began their invasion with a brief biotrophic phase in which they attacked live cells before transitioning into a longer necrotrophic phase in which they multiplied amid dead cells. Acervuli emerged as a stroma immediately under the outer periclinal epidermal walls after the cortical tissue was substantially disturbed.

Detection and identification. *Colletotrichum* species have traditionally been classified based on morphological characteristics such as conidial and apressorium morphology,

pathogenicity tests, and physiological and biochemical techniques (Mun˜oz et al., 2000).
Molecular genetic research is helping to understand the systematics of the genus *Colletotrichum*. Isozyme comparison, mitochondrial DNA restriction fragment length polymorphisms (mtDNA RFLPs), arbitrarily primed polymerase chain reaction (PCR), and ribosomal DNA (rDNA) restriction analyses have managed to make the most significant contributions to the molecular characterization of Colletotrichum species causing strawberry anthracnose (Martinez-Culebras et al., 2003).

Management. Any cultural practice that reduces rain splash, such as spreading straw mulch around the margins of and between rows of plants, would assist. Only a few cultivars have substantial anthracnose tolerance. Day-neutral types are more vulnerable. Because inoculum may be transported about on harvesting equipment and harvesters' hands, it makes sense to begin operations in less severely afflicted regions before moving on to locations where the disease is more severe. Low tunnels significantly reduce anthracnose occurrence, whereas high tunnels almost eradicate fruit symptoms even without the application of fungicides, making them very valuable for organic producers (Kathy & Timothy, 2019).
Anthracnose in strawberries has been controlled substantially with germicide operations. Since the 1990s, the most generally used pesticides have been benzimidazole pesticides similar as carbendazim and thiophanate methyl (Zhang et al., 2020).
Control of *C. acutatum* is heavily reliant on regular and many treatments of multi- and single-site fungicides. Multisite fungicides, such as captan, provide adequate control of *C. acutatum* but have no curative effect, necessitating weekly treatments throughout the season (Forcelini & Peres, 2018).
Early in the season, azoxystrobin (Quardris®), boscalid + pyraclostrobin (Pristine®), and cyprodinil + fludioxonil (Switch®) consistently offered efficient anthracnose control. Before transplants are planted in producing fields, they should be dipped in fungicides or rinsed with clean water to remove spores or other propagules from the planting stocks, allowing disease management (Daugovish et al., 2009).
Biocontrol using beneficial microorganisms like *Streptomyces* has been used successfully to control fruit postharvest diseases. In a research by Li et al. (2021), strain H4 shown strong antifungal activity against *C. fragariae*, which was isolated from *Dichotella gemmacea* in the South China Sea's Xisha islands. A preventative therapy based on strain H4 extracts greatly decreased the severity and incidence of anthracnose disease while also preserving the hardness and color of harvested strawberry fruits. In vitro, extracts efficiently inhibited *C. fragariae* mycelial growth and spore germination. Pathogenic fungi's mycelial structure showed deformation, shrinkage, collapse, and tortuosity.
Trichoderma is also known to be effective in controlling certain plant pathogens. For instance, in experiments conducted with TRICHODEX, various isolates of the fungus were able to control anthracnose and grey mould in strawberry (Freeman et al., 2004).
Li et al. (2021) discovered that strain QN1NO-4 obtained from noni (*Morinda citrifolia* L.) fruit has excellent antifungal activity against *C. fragariae*. Strain QN1NO-4 belongs to the genus *Bacillus* based on its physicochemical characteristics and phylogenetic tree of the 16S rRNA genome. The average nucleotide identity (ANI) computed by comparing two standard strain genomes was less than 95-96 percent, indicating that the strain is an unique *Bacillus* species designated *Bacillus safensis* sp. QN1NO-4. Strawberry anthracnose of harvested fruit was substantially decreased by its extract.
Based on research findings, it can be inferred that thyme, cinnamon bark, and clove bud essential oils exhibit antifungal properties against *C. acutatum*. In disease-friendly settings, thyme EO volatiles fully prevented pathogen penetration and growth on strawberry fruit, whereas cinnamon bark EO volatiles decreased pathogen penetration and development. As a result, thyme and cinnamon bark may be evaluated as promising fumigant options in the management of strawberry anthracnose (Duduk et al., 2015).
To reduce anthracnose in strawberries, a minimum of nitrogen and potassium should be

included in any fertilizer used, with no more than 15 and 10 units of nitrogen and potassium, respectively. On average, strawberry plants treated with ammonium nitrogen had more disease than those treated with nitrates, but the differences were not statistically significant (Nam et al., 2006).

CONCLUSIONS

The qualities of strawberries: special taste and aroma, their high content of vitamin C, suitability for home processing, as well as impressive ecological adaptability, given the high potential for genetic variability, have led to a large expansion of cultivated areas with strawberries worldwide. The expansion of cultivated areas and the widespread use of chemicals have created problems in the context of the management of the main diseases of the strawberry crop. Therefore, the solution to these problems is integrated control, which aims to reduce the number of chemical treatments, the use of low-toxic and selective pesticides, and the use of biological methods.

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