RESEARCH
www.commprac.com
ISSN 1462 2815

AIRBORNE AND FOLIAR FUNGAL FLORA OF VARIOUS STRAWBERRY VARIETIES GROWN IN GHARB- LOUKKOS (MOROCCO)

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DOI: 10.5281/zenodo.13304338

Abstract

The objective of this study is to determine the fungal flora present in the air and on the foliar parts of strawberry plants, varieties Festival and Camarosa, cultivated in the Moulay Bouselham region (North-ouest Morocco), and to highlight the influence of microclimate on their development. Surveys were carried out on three farms under irrigated shelter, where microclimatic conditions, such as temperature and relative humidity, were measured and analysed. The results reveal significant fungal diversity, influenced by microclimatic conditions. In fact, variations in temperature and relative humidity have a significant influence on the distribution of fungal species. *Botrytis cinerea* reached high levels of contamination, with 8712.7 CFU/m² in MV1 and 8492.6 CFU/m² in GV1. *Cladosporium cladosporioides* was also very present, with values reaching 17,802.9 CFU/m² in SV3 and 10,788.7 CFU/m² in MV1, while *Fusarium oxysporum* showed a more limited presence, with values of 440.3 CFU/m² in GV2 and 377.4 CFU/m² in GV3. Temperatures were ranged from 17.44°C to 28.26°C, and relative humidity between 50.96% and 61.26%. These climatic conditions favoured fungal contamination. *Botrytis cinerea* was very present in the leaves of GV1 (90%) and MV1 (82%), while *Podosphaera aphanis* was dominant in MV3 (56%) and GV3 (56%). *Cladosporium cladosporioides* was strongly present in MV3 (49%) and SV2 (45%). These results highlight the importance of microclimate management and agricultural practices in reducing losses due to fungal diseases in strawberry cultivation.

Keywords: Fungal Flora, Air, Strawberry Plants, Temperature, Humidity, Diseases, Morocco.

1. INTRODUCTION

Strawberry is a perennial plant native to America and belonging to the rosacea family. It has been introduced in Morocco since 1930 as part of tests to diversify domestic production. Its extension has only begun since 1980 mainly in the irrigated areas of Loukkos and Gharb. The area is currently around 3,300 ha, 80% of which is located in Loukkos. National production exceeds 130 thousand tons/year (1). Several varieties were planted in 2013-2014: Festival, San Andrea, Fortuna, Splendor, Benicia, Camarosa, Sabrina. With a dominance of Camarosa and Festival at the level of both regions. Two types of seedlings are used by farmers, namely mound seedlings and bare-root seedlings, which remain the most abundant. In addition, all farmers use fresh seedlings (2). Strawberry culture, like any culture, can encounter several phytosanitary problems, it can be attacked by viruses (3, 4), bacteria (5, 6, 7), mites (8, 9), nematodes (10), pests (11), weeds (12) and fungi (13). Strawberry fungal diseases cause considerable economic losses in growing areas around the world (14). The main diseases encountered are oidium, botrytis, anthracnose and mildew. Oidium and Botrytis remain the two most common and dangerous diseases as they can spoil production throughout the year if adequate measures are not taken in time. Oidium is *a* limiting factor in the production of strawberries (*Fragaria×ananassa* Duch.) worldwide (15). The disease can be particularly problematic when strawberries are

produced in greenhouses or polyethylene tunnels, both of which are conducive to severe epidemics of oidium (16), *Botrytis cinerea* can also cause losses before and after harvest. Botrytis *cinerea* disease is of major economic importance in several berry crops, including strawberry, raspberry and grape (17). In the Gharb - Loukkos region, pest management is becoming increasingly complex due to their adaptation to agricultural practices and climate change. Farmers therefore need to consider many factors and have more information to make the best decision. Aerial dissemination is one of the propagation mechanisms used by phytopathogenic fungi to reach susceptible plants in the same field or in a neighbouring field. This short-range dispersion proves to be paramount in the development of a large number of diseases of economic importance. Moreover, it is to control these aerial dispersal diseases that agricultural producers use the greatest number of fungicide applications. Thus, the objective of this study is to determine the aerospace and foliar fungal flora of some strawberry varieties grown in the Gharb- Loukkos region (Morocco) and to demonstrate the influence of microclimate on the development of this fungal flora.

2. MATERIAL AND METHODS

2.1. Prospecting and data collection

Three visits (V1, V2 and V3) were conducted to three villages in the vicinity of Moulay Bouselham, located north of the Atlantic coast of Gharb and south of Loukkos (70 km north of Kenitra and 35 km south of Larache) February 4 (V1), March 3 (V2) and April 24 (V3) 2014 in three strawberry farms under irrigation with the tasting system on sandy soils:

- A large farm (G) of 32 hectares consisting of 9 large greenhouses cultivated by the Festival variety in Dlalha (in the second year of cultivation).
- An average exploitation (M) of eight hectares under Nantes tunnels cultivated by the Festival variety in Aït Aguile (in the second year of cultivation).
- A small farm (S) of two hectares under Nantes tunnels cultivated by the Camarosa variety in Gnafda (in the first year of cultivation).

The collection of data on its three farms was done using a fact sheet containing different variants (Figure 1). The files were completed on site with the managers of these farms. Diagonal sampling was carried out for the different parameters and samples.

2.2. Measurement of temperature and relative humidity

In order to determine the influence of microclimate on the development of aerospace and leaf fungal flora at the culture level, temperature and relative humidity were measured within tunnels. These parameters were taken at each visit at the level of the three farms. These measurements were recorded using a testo 625 thermo-hygrometer, five repetitions were carried out in each plot.

2.3. Estimation of aerospace mycoflora

In each plot visited, the lids of five Petri dishes containing the PDA medium (40 g potato dextrose agar, 5 g agar-agar, 1000 ml distilled water) supplemented with 50 mg chloramphenicol are removed. The bottoms of boxes containing the culture medium shall be placed on the mulch and exposed to the ambient air of the greenhouse or tunnel of each holding for 5 minutes. The boxes are then covered with their lids, sealed

with parafilm, numbered, deposited in a portable cooler and brought back to the laboratory for analysis. After incubation at 28 °C. And in darkness for 7 days, a counting of the CFU (colony forming unit) is done by marking a cross on the back of the Petri dish in a surface of 63.6 cm2and a height of 10 mm. Each colony is purified by several transplants on PDA medium and identified under an optical microscope using the determination keys (18, 19, 20, 21, 22).

2.4. Estimate of foliar mycoflora

In each plot visited, samples of healthy-looking strawberry plants are randomly taken from five points on the diagonal, placed in plastic bags and labelled. They are then brought back to the laboratory for examination. Samples that cannot be examined directly are deposited in the cold room for further study. Analysis of mycoflora associated with the aerial portion of healthy seedlings (leaves, stems and sepals) is carried out using the modified Buvard method (23). The fragments of the leaves of 1 cm^2 and the stems of 1 cm in length are washed with tap water, disinfected with sodium hypochlorite at 1% for five minutes and then rinsed with sterile distilled water.

They are then placed sterilely in Petri dishes containing two washers of filter paper, previously sterilized and then moistened with sterile distilled water. The dishes are then incubated at 22 ° C. in continuous light for seven days. The fragments are then examined under an optical microscope in order to observe the presence of fungal fructifications. The conidia detected are transferred aseptically under a microscope, using a stretched glass capillary, previously sterilized with the flame and cooled in the culture medium. They are subsequently deposited and dispersed on the surface of the agar medium (15 g of Agar-Agar, 1000 ml of distilled water), then transferred again with a part of the agar medium using a needle sterilized on the surface of the PDA medium. Each fragment was placed in a test tube containing 1 ml of sterile distilled water stirred in the vortex for one minute to detach the conidia from the mycelial fragments, the conidian suspensions being adjusted with sterile distilled water so as to obtain a final concentration of 10^5 spores/ml. Thus, the number of conidia was determined using a Malassez cell under an optical microscope at magnification × 400 and expressed as the number of conidia/cm2. The identification of the fungal species is carried out using the determination keys (18, 19, 20, 21, 22). The percentage of infection and/or contamination by the different fungal species is calculated according to the Ponchet method (24) which defines the frequency of isolation of the different fungi from 100 lesions or 100 rots present on the plants studied according to the equation:

$$PC = (NFI/ NTF) \times 100$$

Where **PC** is the percentage of infection and/or contamination; **NFI** is the number of fragments infected with a given fungal species and **NTF** is the total number of healthy fragments.

The isolation frequency of each fungal species from air is estimated by the number of colony-forming units (CFU) per square meter.

2.5. Statistical analysis

Data from all measured parameters were analyzed for variance and significant differences between means were evaluated using the lowest significant difference method at $P < 0.05$ (least significant difference (LSD) test), a test to compare averages is applied to the data.

3. RESULTS

The analysis of the fact sheets completed by the managers of the three farms yielded data on the previous crop, fumigation and fungicide treatment, while the measurement of the climatic conditions was recorded by ourselves. According to the study, the Festival variety was grown on the large and medium plots for two consecutive years, while the farmers of the small plot cultivated the Festival variety before replacing it with the Camarossa variety. All farmers also performed fungicide treatment during the same study week. On the large plots, six different commercial products were used, two on the medium plots and one on the small plots. Most of these products belong to the chemical family of nitrogen heterocycles and are used to control oidium and grey rot (Table 1).

During the months of February, March and April, temperature measurements were made in the air of the fields visited. The data collected reveal significant variations in temperatures between the different stations (Figure 2). In analyzing these data, we can see that temperatures vary between stations for each month. Station GV2 recorded the highest temperature in March at 28.26 ° C, while Station SV1 recorded the lowest temperature in February at 17.44 ° C. For April, station MV3 recorded the highest temperature at 26.14 ° C, while station GV3 recorded the lowest temperature at 23.62 ° C. As for the relative humidity of the air (Figure 3), in March Station GV2 recorded the highest relative humidity at 50.96%, while in February Station MV1 recorded the lowest relative humidity at 52.46%. In April, station GV3 recorded the highest rate at 61.26%, while station SV1 recorded the lowest relative humidity at 60.12%.

Microscopic observation of colonies has identified some species such as *Botrytis cinerea, Podosphaera aphanis, Cladosporium cladosporioides, Aspergillus nidulans, Rhizopus stolonifer, Fusarium oxysporum, Phytophthora* spp., *Alternaria alternata* and *Colletotrichum acutatum* (Figure 4). Averages of colony forming units during February, March, and April in the three plots were calculated (Table 2).

The results show great variability in the distribution and concentration of different fungal species in the plots studied. Some species, such as *Botrytis cinerea*, *Cladosporium cladosporioides*, and *Aspergillus nidulans*, have very high levels in some plots. *Botrytis cinerea* had significant values of 8712.7 CFU/m² in MV1 and 8492.6 CFU/m² in GV1. *Cladosporium cladosporioides* reached 17802.9 CFU/m² in SV3 and 10788.7 CFU/m² in MV1*. Aspergillus nidulans* have values of 7674.8 CFU/m² in MV1 and 7517.4 CFU/m² in GV1, indicating areas potentially favourable to their development. Other species show a more limited presence. *Fusarium oxysporum* has values of 440.3 CFU/m² in GV2 and 377.4 CFU/m² in GV3. *Phytophthora spp.* has levels of 251.6 CFU/m² in GV3. *Alternaria alternata* at 62.9 CFU/m² in MV3 and GV3, while Colletotrichum *acutatum* showed a concentration of 157.26 CFU/m² in GV1.

The results given in the tables: leaves (Table 3), stems (Table 4) and sepals (Table 5) show that the frequency of isolation of the fungal species depends on the source of isolation. The results show significant variability in fungal contamination of strawberry leaves in the plots studied (Table 3). *Botrytis cinerea* is very present in parcels GV1 (90%), MV1 (82%) and GV2 (54%), but weak in MV3 (16%) and SV3 (20%). *Podosphaera aphanis* has high levels in MV3 (56%) and GV3 (56%), but remains low in SV1 (9%) and SV2 (12%). *Cladosporium cladosporioides* are strongly present in MV3 (49%) and PS2 (45%), but very low in SV1 (3%) and MV1 (3%). *Alternaria*

alternata showed a peak of contamination in SV3 (5%), with low levels uniform elsewhere (0-2%*). Pestalotia longisetula* reaches its highest levels in MV2 (5%) and MS3 (7%), but is absent in SV1, GV1, GS2 and GV3 (0%). *Fusarium oxysporum* is high in SV3 (3%) and absent in most plots. *Rhizopus stolonifer* shows high levels in GV1 (9%), GV2 (8%) and MV2 (8%), but low levels in SV3 (1%) and MV3 (3%).

The results in Table 4 show significant variability in fungal contamination of strawberry stems in the plots studied. *Botrytis cinerea* is particularly present in GV1 (75%) and MV1 (69%), but weak in MV3 (9%) and SV3 (12%). *Podosphaera aphanis* has high levels in MV3 (59%) and SV3 (31%), but remains low in SV1 (8%) and MV2 (6%). *Cladosporium cladosporioides* are strongly present in GV3 (46%) and SV3 (37%), but very low in MV1 (2%) and MV2 (2%). *Alternaria alternata* shows a uniformly low contamination rate, with a peak of 2% in GV2. *Pestalotia longisetula* reaches its highest level in PS3 (3%), but is absent in most plots. *Fusarium oxysporum* is present uniformly at a low rate, reaching 1% in several plots. *Rhizopus stolonifer* shows varying levels, reaching a peak of 3% in GV2, but remaining low or absent elsewhere.

The results in Table 5 show significant variability in fungal contamination of strawberry sepals in the plots studied. *Botrytis cinerea* is very present in parcels GV1 (80%), MV1 (72%) and SV1 (68%), but weak in MV3 (6%) and SV3 (13%). *Podosphaera aphanis* has high levels in GV3 (45%) and MV3 (40%), but remains low in SV1 (9%) and MV1 (12%). *Cladosporium cladosporioides* are strongly present in GV3 (43%) and SV2 (28%), but absent in MV2 (0%) and GV2 (0%). *Alternaria alternata* shows a low and uniform contamination rate, with a peak of 2% in MV3. *Pestalotia longisetula* reaches its highest level in MV3 (7%), but is absent in most other plots. *Fusarium oxysporum* has low levels, reaching 1% in SV3, MV3, and GV1. *Rhizopus stolonifer* shows varying levels, peaking at 4% in SV1, but remaining low or absent in other plots.

The results in Table 5 also show significant variability in fungal contamination of strawberry sepals in the plots studied.

In addition, *Botrytis cinerea* is highly present in parcels GV1 (80%), MV1 (72%) and SV1 (68%), but shows low levels of contamination in MV3 (6%) and SV3 (13%). *Podosphaera aphanis* has high levels in GV3 (45%) and MV3 (40%), but remains low in SV1 (9%) and MV1 (12%). *Cladosporium cladosporioides* are strongly present in GV3 (43%) and PS2 (28%), but are absent in MV2 (0%) and GV2 (0%). *Alternaria alternata* shows a low and uniform contamination rate, with a peak of 2% in MV3*. Pestalotia longisetula* reaches its highest level in MV3 (7%), but is absent in most other plots. *Fusarium oxysporum* has low levels, reaching 1% in SV3, MV3, and GV1. *Rhizopus stolonifer* shows varying levels, peaking at 4% in SV1, but remaining low or absent in other plots.

The number of spores is determined using a Malassez cell under microscope for the different species encountered (*Botrytis cinerea*, *Podosphaera aphanis*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Fusarium oxysporum*, Pesttigaotia longisetula*, and Rhizopus stolonifer*) on the different fragments of the leaves.

Podosphaera aphanis shows high levels in GV1 (2833×10^5 spores/cm²) and SV1 (2028.6×10^5spores/cm²), but remains low in MV3 (786.2×10^5 spores/cm²) and SV2 (1119.4×10^5 spores/cm²). *Cladosporium cladosporioides* show high contamination in SV3 (8406.4×10^5 spores/cm²) and GV3 (7319.8×10^5 spores/cm²), but very low levels in MV1 (386.4×10^5 spores/cm²) and SV1 (386.6×10^5 spores/cm²). *Alternaria alternata* has uniform low levels, with a peak in GV1 (253.2×10^5 spores/cm²) and low

levels in MV2 (0 × 10^5 spores/cm²) and GV2 (19.8 × 10^5 spores/cm²). *Pestalotia longisetula* reaches high levels in MV3 (8706.4 × 10^5 spores/cm²) and MV2 (5473 × 10^5 spores/cm²), but is absent in GV1, GV2 and GV3. *Fusarium oxysporum* is higher in PS3 (846.6 × 10^5 spores/cm²), with low or zero levels in the majority of plots. *Rhizopus stolonifer* has a strong presence in MV3 (3346.4 × 10^5 spores/cm²) and GV3 (2373.6 × 10^5 spores/cm²), but is absent in most other plots.

These results show significant variability in fungal contamination of strawberry stems across plots and species.

Botrytis cinerea showed high levels of contamination in GV1 (8226.2 × 10^5 spores/cm²) and MV1 (1986.2 × 10^5 spores/cm²), while the lowest levels were observed in GV3 (6.6 × 10^5 spores/cm²) and MV3 (79.8 × 10^5 spores/cm²*). Podosphaera aphanis* has high levels in GV1 (2093 × 10^5 spores/cm²) and GV2 (1733 × 10^5 spores/cm²), but the lowest levels are in SV2 (546.4 × 10^5 spores/cm²) and MV2 (533 × 10^5 spores/cm²*). Cladosporium cladosporioides* are highly contaminated in GV3 (4373.2 × 10^5 spores/cm²) and SV2 (3379.6 × 10^5 spores/cm²), while the lowest levels are observed in MV1 (399.6 × 10^5 spores/cm²) and SV1 (626.4 × 10^5 spores/cm²). *Alternaria alternata* shows uniform low levels, with a peak in GV2 (153 × 10^5 spores/cm²) and the lowest levels in MV1 (6.6 ×10^5 spores/cm²) and MV2 (6.6 × 10^5 spores/cm²). *Pestalotia longisetula* reaches its highest level in MV3 (4893 × 10^5spores/cm²), while it is completely absent in several plots (SV2, SV3, GV1, GV2, and GV3). *Fusarium oxysporum* shows high levels in SV3 (279.8 × 10^5 spores/cm²) and GV1 (153.2 × 10^5 spores/cm²), but the lowest or zero levels are observed in the majority of the plots. *Rhizopus stolonifer* has a low presence only in SV3 (33.2 × 10^5 spores/cm²) and is absent in all other plots.

The results of Table 8 also show significant variability in fungal contamination of the sepals of strawberry flowers grown in the Moulay Bousselham area. This variability is similar to that observed in stems, with varying levels of fungal contamination across plots and species. *Botrytis cinerea* showed high levels of contamination in GV1 (2053 × 10^5 spores/cm²) and MV1 (1779.8 × 10^5 spores/cm²), while the lowest levels were observed in SV3 (26.6 × 10^5spores/cm²) and MV3 (106.6 × 10^5 spores/cm²). *Podosphaera* aphanis shows high levels in GV1 (2306.4 × 10^5 spores/cm²) and MV2 (1386.4 × 10^5 spores/cm²), but the lowest levels are in SV3 (133.2 × 10^5 spores/cm²) and GV3 (173.2 × 10^5 spores/cm²). *Cladosporium* cladosporioides are highly contaminated in GV3 (4786.4 × 10^5 spores/cm²) and SV2 (2706.4 × 10^5 spores/cm²), while the lowest levels are observed in MV1 (40 × 10^5 spores/cm²) and SV1 (659.8 × 10^5 spores/cm²). *Alternaria alternata* showed uniform low levels with a peak in MV3 and GV3 (40 × 10^5 spores/cm²), and zero levels in several plots (SV2, MV1, MV2, GV1, GV2). *Pestalotia longisetula* reaches its highest level in MV3 (6246.4 × 10^5 spores/cm²), while it is completely absent in several plots (SV1, GV1, GV2, GV3). *Fusarium oxysporum* shows high levels in GV1 (113.2 × 10^5 spores/cm²), but the lowest or zero levels are observed in the majority of plots. *Rhizopus stolonifer* has a strong presence only in SV3 (233.2 × 10^5 spores/cm²) and GV3 (253.2 × 10^5 spores/cm²), but is absent in all other plots.

Release date : .....
Plot (farm): .....
What is the previous culture ?.....
Did you fumigate the soil before culture ? Yes ☐ No ☐
If yes, what product was used ?.....
Did you apply a fungicide treatment ? Yes ☐ No ☐
If yes, since when ?.....
What products are used ?.....

♦ **Measuring climatic conditions :**

samples	physical support	parameters	Max	Hold	Min
1	Air	H.R			
		T°			
2	Air	H.R			
		T°			
3	Air	H.R			
		T°			
4	Air	H.R			
		T°			
5	Air	H.R			
		T°			

FIG. 1. Information sheet

Table 1: List of Fungicides used by farmers in the three plots prior to release.

Fungicide	Active ingredient	Chemical family	Mode of action	Pathogen	G	M	P
Teldor 50 WG	Fenhexamid	Hydroxy- anilides	Acts by inhibiting the elongation of germ tubes and mycelial hyphae. Contact, Preventive	*Botrytis cinerea*	x	x	x
Switch	Cyprodinil+ Fludioxonil	Anilino - pyrimidines+ Phenyl-pyrroles	Acts by inhibition of spore germination and elongation of germ tubes and mycelial hyphae. Systemic, preventive and curative. Contact, preventive	*Botrytis cinerea*	x	x	x
Flint 50 WG	Trifloxystrobine	Strobilurins	Affects respiratory processes. Penetrating, preventive, curative and eradicating.	Powdery mildew	xx	x	xx
Stroby WG	Kresoxim-méthyl	Strobilurins		Powdery mildew	x	x	x
Bayfidan 312 SC	Triadiménol	Triazoles	Acts on the biosynthesis of sterols, without effect on oomycetes. Systemic, preventive, curative and eradicating	Powdery mildew	x		x
Systhane 240 EC	Myclobutanil	Triazinones		Powdery mildew	x	x	x
Signum WG	Boscalid+ Pyraclostrobine	Nicotinamides+ Morpholines	Acts on respiratory processes by inhibiting mitochondrial complex II in the respiratory chain. Penetrating, preventive. Acts by inhibiting lipid synthesis. Contact, penetrating, preventive, curative and eradicating	*Botrytis cinerea+ Powdery mildew*	xx		xx

Table 2: Means of colony forming units per square metre of fungal species identified in Petri dishes exposed to ambient air from the three plots visited during the months of February, March and April 2014 (expressed in CFU/m ²).

Fungal species	Farm / Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	1918,6^{d}	5661,7^{b}	3019,5^{c}	8712,7^{a}	4214,8^{c}	1321,1^{e}	8492,6^{a}	1729,9^{d}	2138,8^{d}
Podosphaera aphanis	912.2^{b}	440.3^{b}	3019.5^{a}	1761.4^{ab}	550.4^{b}	1761.4^{ab}	943.5b	471.8^{b}	1352.5 ^{b}
Cladosporium cladosporioides	1950,1^{d}	3742,9^{c}	17802,9^{a}	10788,7^{b}	3428,5^{c}	4529,4^{c}	5630,2^{b}	342,8^{e}	1100,8^{d}
Aspergillus nidulans	1918,7^{e}	6416,6^{b}	6070,6^{c}	7674,8^{a}	5410,1^{c}	6416,6^{b}	7517,4^{a}	723,4^{f}	1824,3^{d}
Rhizopus stolonifer	2075,9^{e}	8775,6^{b}	5441,5^{c}	10694,3^{a}	3680,1^{d}	6290,8^{c}	6982,8^{b}	660,5^{f}	2516,2^{e}
Fusarium oxysporum	0^{d}	188,7^{c}	125,8^{c}	0^{d}	345,9^{b}	62,9^{c}	94,3^{c}	440,3^{a}	377,4^{a}
Phytophthora spp.,	0^{d}	0^{d}	157,3^{b}	157,3^{b}	157,3^{b}	62,9^{c}	0^{d}	0^{d}	251,6^{a}
Alternaria alternata	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	62,9^{a}	0^{b}	0^{b}	62,90^{a}
Colletotrichum acutatum	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	157,26^{a}	0^{b}	0^{b}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

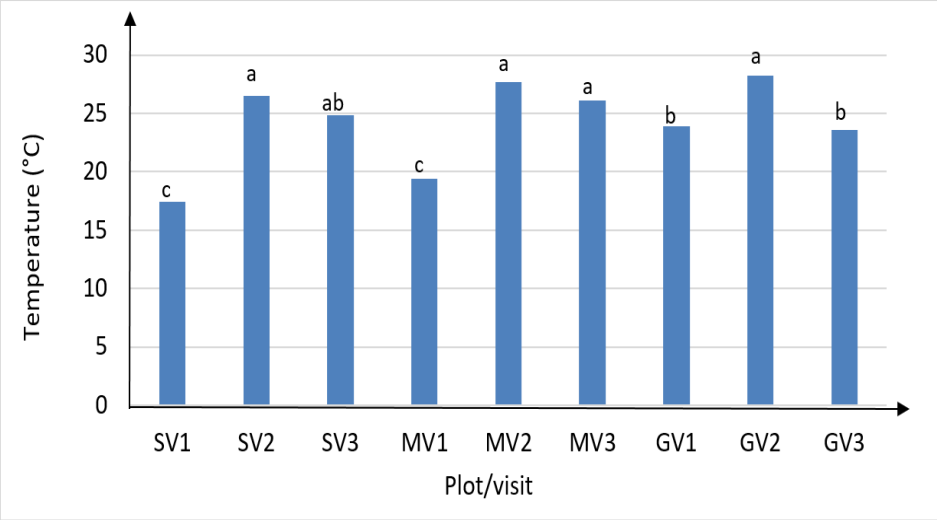

Fig 2: Values of the air temperature of the three farms during the three visits.

SV1, SV2 and SV3: three measurements made separately in February (SV1), March (SV2) and April (SV3) on the Gnafda plot.

MV1, MV2 and MV3: Measurements carried out in February (MV1), March (MV2) and April (MV3) on the Dlalha plot.

GV1, GV2et GV3: The measurements carried out in February (GV1), March (GV2) and April (GV3) on the parcel of Ouled Aguile.

The bars with the same letter show no significant difference at the 5% probability level by the LSD test.

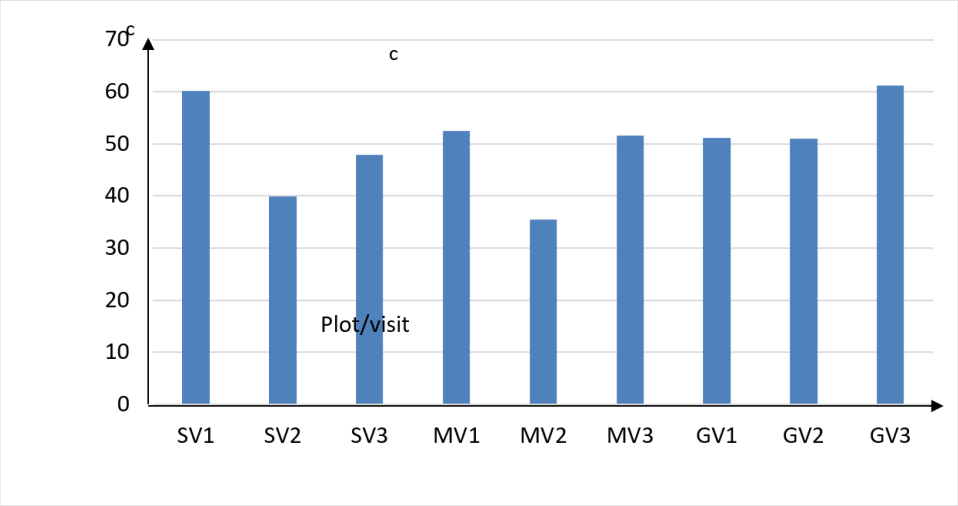

Fig 3: Percentage of relative humidity of the air of the three stations during the three visits.

SV1, SV2 and SV3: three measurements made separately in February (SV1), March (SV2) and April (SV3) on the Gnafda plot.

MV1, MV2et MV3: Measurements carried out in February (MV1), March (MV2) and April (MV3) on the Dlalha plot.

GV1, GV2et GV3: The measurements carried out in February (GV1), March (GV2) and April (GV3) on the parcel of Ouled Aguile.

The bars with the same letter show no significant difference at the 5% probability level by the LSD *test.*

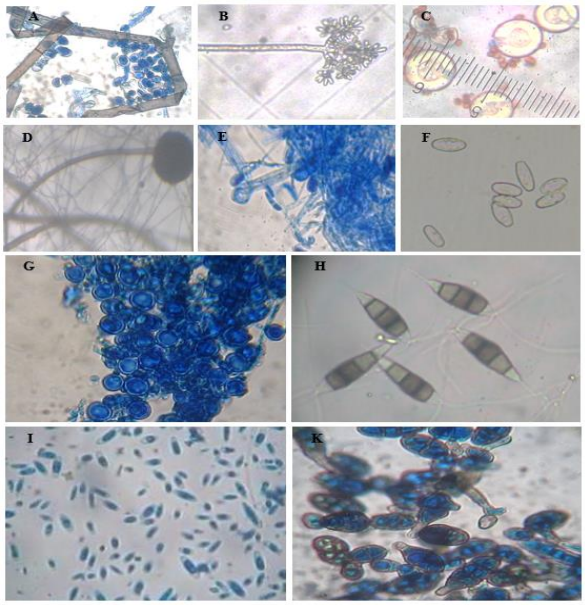

Fig 4: Microscopic appearance of fungal species isolated from various vegetative organs of the strawberry plant : *Botrytis cinerea* (A), *Cladosporium cladosporioides (B), Aspergillus nidulans (C), Rhizopus stolonifer(D), Fusarium oxysporum (E), Podosphaera aphanis* (F), *Phytophtora* spp *(G), Colletotrichum acutatum (H), Pestalotia longisetula* (*I), Alternaria alternata (K).*

Table 3: **Isolation frequencies of fungal species contaminating leaves of strawberry plants grown in plots located at the Moulay Bousselham area during the February to April 2014 study period (expressed as percent contamination/infection%).**

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	46^{c}	25^{d}	20^{d}	82^{a}	43^{c}	16^{d}	90^{a}	54b	21^{d}
Podosphaera aphanis	9^{d}	12^{d}	42^{b}	15^{d}	20^{c}	56^{a}	19^{c}	22^{c}	56^{a}
Cladosporium cladosporioides	3^{e}	45^{b}	40^{b}	3^{e}	18^{c}	49^{a}	16^{c}	7^{d}	44^{b}
Alternaria alternata	1^{b}	2^{b}	5^{a}	1^{b}	0^{b}	2^{b}	2^{b}	1^{b}	1^{b}
Pestalotia longisetula	0^{c}	3^{b}	3^{b}	1^{b}	5^{a}	7^{a}	0^{c}	0^{c}	0^{c}
Fusarium oxysporum	0^{b}	1^{b}	3^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Rhizopus stolonifer	7^{b}	5^{b}	1^{c}	7^{b}	8^{a}	3^{c}	9^{a}	8^{a}	2^{c}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

Table 4: Isolation Frequencies of Fungal Species Contaminating Strawberry Plant Stems Grown in Parcels Located at the Moulay Bousselham Area during the February to April 2014 Study Period (expressed as percent contamination / infection %).

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	57^{b}	27^{c}	12^{d}	69^{b}	38^{c}	9^{d}	75^{a}	49^{b}	12^{d}
Podosphaera aphanis	8^{d}	11^{d}	31^{b}	15^{c}	6^{d}	59^{a}	19^{c}	26^{b}	17^{c}
Cladosporium cladosporioides	6^{c}	33^{b}	37^{b}	2^{c}	2^{c}	27b	6^{c}	6^{c}	46^{a}
Alternaria alternata	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	1^{b}	0^{b}	2^{a}	1^{b}
Pestalotia longisetula	1^{b}	0^{b}	3^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
Fusarium oxysporum	0b	1a	1a	0b	1a	1a	1a	0b	0b
Rhizopus stolonifer	1^{b}	2^{a}	0^{c}	2^{a}	1^{b}	1^{b}	0^{c}	3^{a}	1^{b}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

Table 5: Isolation frequencies of fungal species contaminating the sepals of strawberry plants grown in plots located at the Moulay Bousselham area during the February to April 2014 study period (expressed as percent contamination/infection%).

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	68^{ab}	24^{c}	13^{c}	72^{a}	34^{bc}	6^{d}	80^{a}	45^{b}	13^{c}
Podosphaera aphanis	9^{f}	13^{e}	29^{c}	12^{e}	17^{d}	40^{b}	18^{d}	31^{c}	45^{a}
Cladosporium cladosporioides	6^{d}	28a	23^{b}	6^{d}	0^{e}	21^{b}	11^{c}	0^{e}	43^{a}
Alternaria alternata	1^{a}	1^{a}	0^{b}	0^{b}	0^{b}	2^{a}	0^{b}	0^{b}	1^{a}
Pestalotia longisetula	0^{b}	0^{b}	1^{b}	0^{b}	1^{b}	7^{a}	0^{b}	0^{b}	0^{b}
Fusarium oxysporum	0^{b}	0^{b}	1^{a}	0^{b}	0^{b}	1^{a}	1^{a}	0^{b}	0^{b}
Rhizopus stolonifer	4^{a}	0^{c}	1^{c}	1^{c}	1c	1^{c}	1^{c}	1^{c}	2^{b}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

Table 6: **Mean spores, counted as 10^5 conidia/cm², from strawberry plant leaves grown in the Moulay Bousselham area plots were measured during the study period from February to April 2014.**

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	1513,2^{b}	739,6^{c}	739,8^{c}	3606,4^{a}	2026,4^{b}	579,8^{c}	4072,6^{a}	2359,8^{b}	2733^{b}
Podosphaera aphanis	2028,6^{b}	1119,4^{c}	1339,6^{c}	1559,6^{b}	1159,6^{c}	786,2^{d}	2833^{a}	2193^{b}	1899,6^{b}
Cladosporium cladosporioides	386,6^{d}	3453^{b}	8406,4^{a}	386,4^{d}	1839,6^{c}	4433^{b}	1258^{c}	3799,4^{b}	7319,8^{a}
Alternaria alternata	126,4^{b}	153,2b	146,4^{b}	33,2^{d}	0^{d}	193^{b}	253,2^{a}	19,8^{d}	40^{c}
Pestalotia longisetula	6,6^{d}	2693^{b}	60^{d}	1786,6^{c}	5473^{b}	8706,4^{a}	0^{e}	0^{e}	0^{e}
Fusarium oxysporum	0^{d}	33,2^{c}	846,6^{a}	13,2^{c}	0^{d}	393,2^{b}	59,2^{c}	0^{d}	0^{d}
Rhizopus stolonifer	0^{c}	0^{c}	39,8^{c}	0^{c}	0c	3346,4^{a}	0^{c}	0c	2373,6^{b}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

Table 7: Mean spores, counted as 10^5 conidia/cm², from strawberry plant stalks grown in the Moulay Bousselham area plots were measured during the study period from February to April 2014.

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	839,6^{c}	299,6^{d}	653^{c}	1986,2^{b}	919,2^{c}	79,8^{d}	8226,2^{a}	3073^{b}	6,6^{d}
Podosphaera aphanis	933,2^{b}	546,4^{c}	906,4^{b}	1039,6^{b}	533^{c}	806,4^{b}	2093^{a}	1733^{a}	986,4^{b}
Cladosporium cladosporioides	626,4^{d}	3379,6^{b}	2759,8^{b}	399,6^{d}	2753,2^{b}	2586,4^{c}	1186,4^{d}	2072,8^{c}	4373,2^{a}
Alternaria alternata	46,6^{c}	53,2^{c}	46,4^{c}	6,6^{d}	6,6^{d}	93,2^{b}	0^{d}	153^{a}	26,6^{c}
Pestalotia longisetula	26,6^{c}	0^{d}	0^{d}	160^{c}	0^{d}	4893^{a}	0^{d}	0^{d}	0^{d}
Fusarium oxysporum	0^{c}	0^{c}	279,8^{a}	0^{c}	0^{c}	0^{c}	153,2^{b}	0^{c}	0^{c}
Rhizopus stolonifer	0^{b}	0^{b}	33,2^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

Table 8: Mean spores, counted as 10^5 conidia/cm², from strawberry plant sepals grown in the Moulay Bousselham area plots were measured during the study period from February to April 2014.

Fungal species	Farm/Visit								
	SV1	SV2	SV3	MV1	MV2	MV3	GV1	GV2	GV3
Botrytis cinerea	866,4^{c}	506,2^{d}	26,6^{e}	1779,8^{b}	1619,6^{b}	106,6^{e}	2053^{a}	1013^{c}	159,8^{e}
Podosphaera aphanis	746,4^{c}	499,6^{d}	133,2^{e}	859,8^{c}	1386,4^{b}	399,8^{d}	2306,4^{a}	806,4^{c}	173,2^{e}
Cladosporium cladosporioides	659,8^{d}	2706,4^{b}	2086,4^{c}	40^{e}	1646,6^{c}	566,6^{d}	726,4^{d}	1419,8^{c}	4786,4^{a}
Alternaria alternata	20^{c}	0^{d}	19,8^{c}	0^{d}	0^{d}	40^{a}	0^{d}	0^{d}	40^{a}
Pestalotia longisetula	0^{d}	413,2^{b}	66,4^{c}	26,6^{c}	26,4^{c}	6246,4^{a}	0^{d}	0^{d}	0^{d}
Fusarium oxysporum	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	20^{b}	113,2^{a}	0^{b}	0^{b}
Rhizopus stolonifer	0^{b}	0^{b}	233,2^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	253,2^{a}

Two results, read on the same line, differ significantly at the 5% threshold, if they are not assigned any letters in common.

4. DISCUSSION

Strawberry cultivation requires precise know-how and careful monitoring. The strawberry prefers a well-ventilated and drained silty, sandy or sandy soil with a pH of 6 to 6.5. It develops particularly well in the sandy to sandy-sandy soils of the Gharb and Loukkos regions, with an optimum pH of 6.5 (Institut National de la Recherche Agronomique, 2022). Belonging to the Rosaceae family, the strawberry needs cold for floral initiation. The ideal temperature for flowering is 10 to 15°C, and a temperature of 20°C with a relative humidity of less than 60% is necessary for optimal fertilization (25).

In this study, strawberry plantations are protected by shelters to gain heat, protect frost blooms, and protect the crop from weather (26, 27). The use of large tunnels improves the productivity and quality of the fruit and prolongs the growing period (28), but their construction is costly. Farmers opt for mini tunnels to reduce costs, reduce losses and increase off-season productivity (29). The leakproofness of tunnels increases the accumulation of heat but prevents the renewal of air, thus increasing relative humidity. Condensation on plastic films improves their ability to withstand heat, but it can also increase cryptogamic diseases and reduce fruit quality (30, 31).

Strawberry cultivation is frequently affected by plant pathogens, requiring regular treatments to maintain quality and yield (15, 32). Farmers on the three plots alternate the use of fungicides against *Botrytis cinerea* and oidium and use strawberry varieties resistant to fungal diseases to meet market requirements (33). Prior to cultivation, they fumigate with metam sodium to control nematodes and soil fungi, which is essential to optimize fruit yield and quality (34). Saksena showed the ability of soil fungi to resist fumigants and recolonize treated soils, which explains the presence of spores of *Aspergillus* and *Phytophthora* as early as February, as well as *Fusarium* and *Alternaria* in some plots from March (35).

Bhatti and Kraft showed that soil moisture increases rhizospheric populations responsible for root wilting and rot (36). Fungal species such as *Rhizoctonia solani, Fusarium oxysporum*, *Macrophomina phaseolina*, and *Colletotrichum* species can

survive long as sclerotia or potential inoculum in plant debris and soil (37, 38, 39, 40, 41, 42, 43).

In this study, the farmers of the large and medium plots cultivated the Festival variety, while those of the small plot chose the Camarosa variety. According to Frang *et al.,* studies show that Camarosa cultivar is more susceptible to pathogens, while Festival cultivar is more resistant (44).

Microscopic observations of colonies have shown that the air contains spores of a few species such as *Botrytis cinerea*, *Podosphaera aphanis***,** *Cladosporium cladosporioides*, *Aspergillus nidulans*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Phytophthora* spp., *Alternaria alternata* and *Colletotrichum acutatum.* These spores are deposited on the different constituents of the plant and when the conditions become favorable will germinate by invading the epidermal cells of the plant.

The study showed significant variation in levels of infestation by different fungi between plots. Medium and large plots have higher infestations for several fungi, including *Botrytis cinerea*, *Aspergillus nidulans*, and *Rhizopus stolonifer*, suggesting more favourable microclimatic conditions and possibly less effective management. The small plot, although less infested with certain fungi such as *Botrytis cinerea*, shows high levels for *Podosphaera aphanis* and *Cladosporium cladosporioides*. These variations can be attributed to specific environmental conditions, management practices and varietal resistance.

This study shows that air temperatures between February and April vary between 17 °C and 28°C in the three plots, with a relative humidity of 44% to 60%. These conditions promote the infection of strawberries with various pathogens. *Botrytis cinerea*, responsible for grey mould, is a global strawberry disease, mainly spread by dead leaves, sick fruits and weeds (45). It often appears on fruit after prolonged periods of wetting, and develops especially after harvest. The production of spores is optimal at temperatures of 15 to 22 °C (46). Spores are released by moisture changes and vibrations caused by wind, rain and insects (15). Infections can occur before harvest and remain latent until storage, where high humidity and low temperature conditions promote their development (47). *B. cinerea* is dispersed mainly by the release of macroconidia into dry air streams (48).

Plants infected with oidium, caused by *Podosphaera aphanis*, are the main sources of inoculum. When conditions are favourable, the conidia produced on these plants are dispersed by the wind. The development and spread of oidium is favoured by high humidity and temperatures between 15 ° C and 27 ° C, while rain, dew and spray irrigation inhibit its development (49).

Burning of strawberry flowers is mainly caused by *Botrytis cinerea*, but *Cladosporium cladosporioides* has also been identified as one of the responsible agents in some fields in California (50). *Cladosporium* species develop optimally at temperatures between 18°C and 28°C, and some can grow at temperatures below 0°C. Most *Cladosporium* species can develop between 30°C and 35°C, but do not survive beyond 35-37°C (51, 52).

Rhizopus rot, caused primarily by *Rhizopus stolonifer*, is a serious post-harvest disease, causing losses during transport and storage of fruit (53). Infected fruit became soft and watery in two to three days, with abundant mycelial mass on the surface and mycelial stolons invading adjacent healthy fruit (53, 54). *R. stolonifer* penetrates

primarily through injury, but enzymes such as cutinase can allow direct penetration of undamaged fruit (55). The fungi *Rhizopus* spp. and *Mucor* spp. naturally occur in soil, plant debris and air. They are dispersed by wind, air currents and some invertebrates. Strawberries close to the ground are more sensitive to soft rot, which is also favored by rain. This disease is more common on strawberries exposed to rain or grown under plastic tunnels, especially along the edges (56). The minimum temperature for spore germination and growth is about 6 °C (15).

Fusarium oxysporum is one of the most virulent telluric pathogens, causing diseases of the collar and roots of strawberries. Its virulence is influenced by the seasonal temperature regime and soil pH. In addition, the severity of the disease varies considerably depending on the fumigated field boards (57) or the type of soil (58). Its presence on the aerial parts of strawberries can be explained by the transmission of soil to leaves and stems by splashing of water during irrigation or rains, as well as by favourable environmental conditions such as moderate temperatures and high humidity.

Wet conditions impede the survival of conidia and sclerotia of *C. acutatum* on strawberry plant debris or in soil (59). At high temperatures of 35 °C with maximum relative humidity, *Colletotrichum fragari*ae infection is more severe than at 25 °C or 30 °C (60). Madden and Boudreau (1997) noted a decrease in the incidence of strawberry anthracnosis with increased plant density *(61). Aspergillus ni*dulans is shown to be a promising antagonist *against Fusarium oxy*sporum and has also shown antagonistic activity against *Colletotrichum gloeospor*ioides, the agent responsible for anthracnosis of *Vanilla plan*ifolia (62, 63).

The wet chamber method favored the development of *Pestalotia longisetula* and *Alternaria alternata* on plant fragments (leaves, stems, and sepals). *Pestalotia longisetula* was detected in small and medium-sized plots in February, March and April. This species has been reported on strawberries in the United States (64), India (65), and recently in Morocco (66). More recently, strawberry isolates have been identified as *Neopestalotiopsis* spp, root pathogens, and strawberry crowns (67, 68). Recent studies have shown that isolates formerly identified as *Pestalotiopsis longisetula* are now classified as *Neopestalotiopsis rosae* (69). The fungus is sometimes isolated with other root and crown pathogens (*Macrophomina phaseolina*, *Colletotrichum* spp., *Phytophthora* spp.). It has long been considered a secondary pathogen with little concern for the strawberry industry, until recently (70).

Alternaria alternata is present on the leaves, stems and sepals of strawberries. Its presence has been reported in Morocco on strawberry (71, 72), olive (73) and banana (74). *Alternaria* species can survive for several years in soil and on plant debris thanks to their melanized mycelium and conidia (75, 76). Chlamydospores also serve as survival structures (77). Spores can germinate in two hours when air is saturated with moisture at temperatures between 8 °C and 32 °C (78). Conidia produce germinative tubes and fibrous mucilage to adhere to the host plant (79). Penetration into plant tissues is carried out through the cells of the epidermis using non-melanized appressoria, and through stomata or wounds (80, 81). Lesions become visible 2 to 3 days after infection, and spore production occurs 3 to 5 days later (82).

5. CONCLUSION

According to this study, a diversified airborne inoculum has been detected, this fungal complex causes very significant damage to strawberry culture and can therefore have an impact on yield if there are no targeted interventions during critical periods. This requires real-time monitoring of aerial inoculum concentrations and well-controlled meteorological variables such as rainfall, relative humidity, mean, minimum and maximum temperature, and wind speed.

The survey carried out among farmers of small, medium and large plots of the Loukkos perimeter revealed that the most worrying symptoms are that of grey rot and oidium, which is why the phytosanitary measures applied by farmers target the pathogens that cause these diseases in order to properly control them throughout their development stages.

To explore the existence of a spatial fungal flora that can colonize cultivated strawberry plants in the Loukkos perimeter, mycological studies have shown the presence of diverse fungal communities in the air and on the various vegetative organs (leaves, stems, and sepals). The isolated fungi belong to five classes: Leotiomycetes, Dothideomycetes, Mucoromycetes, Sordariomycetes and Oomycetes.

The incidence of grey rot was very high in February due to high humidity (between 45% and 61%) and low temperatures (between 17 °C and 25 °C) and that tunnels must remain closed to prolong the production season. While the incidence of oidium was very high in March due to favourable climatic conditions for spore germination: relative humidity (between 37% and 49.5%), temperatures (between 24 °C and 31 °C) and leaves are dry due to tunnel opening.

The high contamination of strawberry soils by telluric fungi is a continuous threat over time and the spore dispersal of these pathogenic fungi occurs through soil, debris from infected plants, water or wind.

To control these fungal species which can have consequences on the health, survival and yield of the plants one can use as alternatives: cultivation practices, biological control, chemical control and use of resistant plants.

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